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REVIEW

The importance of comparative oncology in translational medicine

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Abstract Human cancer is so complex that in vivo preclinical models are needed if effective therapies are to be developed. Naturally occurring cancers in companion animals are therefore a great resource, as shown by the remarkable growth that comparative oncology has seen over the last 30 years. Cancer has become a leading cause of death in companion animals now that more pets are living long enough to develop the disease. Furthermore, more owners are seeking advanced and novel therapies for their pets as they are very much considered family members. Living in the same environments, pets and humans are often afflicted by the same types of cancer which show similar behavior and, in some species, express the same antigen molecules. The treatment of pet tumors using novel therapies is of compelling translational significance.

Keywords Cancer models · Canine tumors · Tumor-associated antigens · Immunotherapy

Abbreviations

AVBC	Australasian Veterinary Boards Council
ACVS	Australian College of Veterinary Scientists
ACVR	American College of Veterinary Radiology
CCR	Center for Cancer Research
CMT	Canine mammary tumors
COP	Comparative Oncology Program
CSPG4	Chondroitin sulfate proteoglycan 4
FDA	Food and Drug Administration
FMT	Feline mammary tumors
GEM	Genetically modified mice
LLO	Listeriolysin
LSA	Lymphosarcoma
MAPK	Mitogen-activated protein kinases
MM	Malignant melanoma
NHL	Non-Hodgkin lymphoma
NHP	Nonhuman primate
NK	Natural killer
NO	Nitric oxide
OSA	Osteosarcoma
PDX	Patient-derived xenografts
SALP	Serum alkaline phosphatase
TAA	Tumor-associated antigen
USDA	United States Department of Agriculture
VCOG	Veterinary Cooperative Oncology Group
VCS	Veterinary Cancer Society

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The complexity of human cancer requires the use of animal models

Cancer models: from the in vitro study of tumor cell lines to in vivo murine models

Cancer is a complex biological process through which a normal cell acquires, step by step, new capabilities that cause its transformation into a tumorigenic and eventually malignant cell. An understanding of this biological complexity has spurred the development of increasingly comprehensive experimental models (Fig. 1).

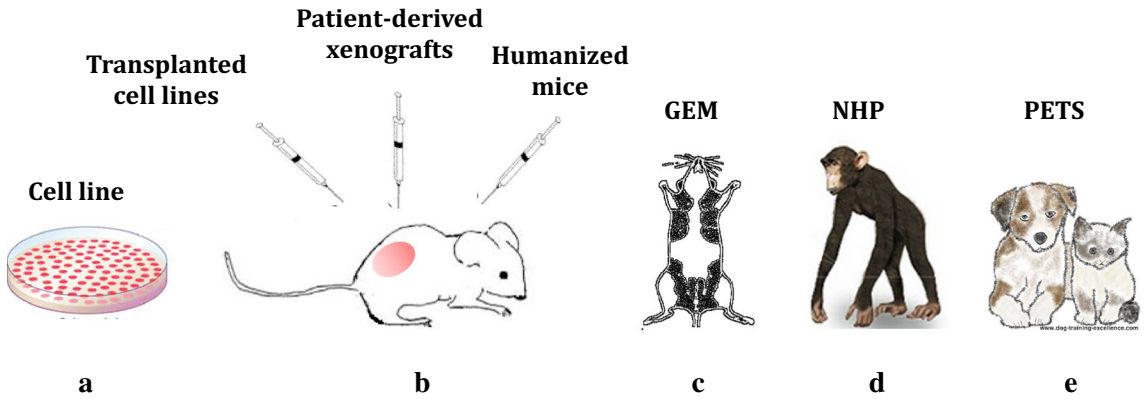

Fig. 1 Evolution of experimental systems toward major complexity and translatability. The study of human cancer complexity has evolved from the use of cancer cell lines (**a**) to the use of ever more complex in vivo systems (**b**, **c**, **d**, **e**). The use of transplantable cancer cell line models, patient-derived xenografts, in syngeneic or immunodeficient mice, and of humanized mice are significant steps toward more comprehensive experimental models (**b**). The advent of genetically engineered mice (GEM) that spontaneously develop tumors and thus recapitulate complete disease evolution provided the first revolution in preclinical cancer research (**c**). To overcome limitations in murine models, testing immunologic therapies in large animals, such as non-human primates (NHPs) which possess immune systems which are closer to ours, has offered advantages in scaling-up doses in human patients (**d**). However, translational medicine research is now rapidly moving toward the study of naturally occurring tumors in companion animals which may be priceless comparative models with which to accelerate the entry of new anticancer therapies into the human sphere (**e**)

For many years, the study of cancer cell lines has been the elective experimental model (Fig. 1a). These lines are a valuable tool for investigating many aspects of cancer biology, such as genetic, epigenetic and cellular pathway alteration, deregulation of proliferation and apoptosis, and for testing therapeutic drugs [1, 2]. Nevertheless, the heterotypic interactions between tumors and the multiple distinct cell types in the microenvironment, including immune cells, are missing in these in vitro studies. The microenvironment evolves in response to tumor survival adaptation, thereby enabling primary, invasive and potentially metastatic growth [3]. This dynamic reciprocity between tumor cells and their environment [4] sculpts the hallmarks of cancer and poses additional challenges in the design of appropriate experimental models.

A relatively easy solution is found in injecting transplantable cancer cell lines into syngeneic or immunodeficient mice [5] (Fig. 1b). These transplantable models can be standardized and provide reproducible data, but remain highly artifactual. They allow the three-dimensional growth of tumors and their direct interaction with the stromal microenvironment to be studied [6]. However, they distort the architectural and cellular complexity of real cancers, as transplanted cells are already transformed and are injected in sufficient number to give rise to a tumor in a young and healthy host [7]. Tumor cells are typically implanted subcutaneously, but implantation into the organ of origin mimics human cancer behavior and the microenvironment more closely. Experimental results generated by orthotopic models are therefore expected to be of higher relevance [8].

A further evolution in transplantable models is found in patient-derived xenografts (PDX) (Fig. 1b). These represent the heterogeneity of human cancers and take into account the natural history of the tumors and/or patients, as regards to (1) inter-patient variability, (2) the diversity of tumor cells with respect to the molecular profile and sensitivity to a specific agent and (3) intratumor heterogeneity. These xenografts derive directly from patient samples, without in vitro manipulation, and provide a more accurate representation of the biological features of human tumors. Moreover, several groups have established disease-specific xenograft panels, directly from patient tumors, which might better reflect clinical responses [9], and help in the selection of the most appropriate drug to be used for that patient. This is an important step forward in personalized medicine, but is not without its pitfalls; implanted patient stroma is replaced little by little by the mouse analog and the mouse immune system is not functional. Both these aspects can affect the translational value of results. Moreover, the overall strategy of implanting a patient tumor into mice (not always successful) and testing drugs on them can be a long, drawn out process which may become a race against time for the patient [10].

The predictive utility of tumor models depends on the fidelity with which they recapitulate the entire evolution of the disease, including interactions between tumors and the immune system, the inherent angiogenic process, tumor-associated fibroblast infiltration and additional stromal components [11]. Genetically modified mice (GEM) which have been engineered to express oncogenes, or in which tumor suppressors have been disrupted, and that

spontaneously develop tumors are a good step forward [5] (Fig. 1c). The relationships between the tumor and the surrounding tissues are preserved, while the progression of carcinogenesis may mimic what is observed in humans [12]. The advent of GEM has revolutionized preclinical cancer research, and several successful preclinical results have been achieved in varying GEM models. Nevertheless, GEM are not devoid of pitfalls: Tumor penetrance is not always complete, meaning very large experimental groups must be used; tumor formation takes longer than in transplantable tumors, thus greatly extending the period of experimental observation; transgene expression is usually under the control of a heterologous promoter, leading to non-physiologic transgene expression in the tissues where that promoter is activated for the entirety of the mouse's life [10]. This may influence the tumor microenvironment and the immune response to the transgene product itself [13, 14]. Mouse models that conditionally express a particular oncogene, in a tissue-specific and time-controlled manner, provide new opportunities to gain insight into the development and treatment of cancer. These conditional mice allow for the study of malignant transformations in the context of an appropriate, non-mutated microenvironment which more faithfully mimics the sporadic nature of human tumors [15–17].

An accurate predictive tumor model should simulate human therapeutic responses and the evolution of resistance. As a consequence, xenografts in mice carrying the human immune system have been proposed as an interesting preclinical model for the in vivo study of the complex interaction between human tumors and the human immune system. Highly immunodeficient mice and transgenic animal models for human factors have been developed and used to generate "humanized mice" [5] (Fig. 1b). However, most existing humanized mouse models cannot develop human innate immune cells, including myeloid cells and NK cells. The literature has recently described two mouse strains, MITRG and MISTRG, in which four human genes that encode cytokines important for innate immune cell development are knocked in their respective mouse loci [18]. Human cytokines facilitate the development and function of monocytes, macrophages and NK cells that are derived from human fetal liver or adult $CD34^+$ progenitor cells transplanted into the mice. Human macrophages infiltrate human tumor xenografts in a manner resembling that of tumors obtained from human patients. The generation of Class I and Class II HLA transgenic NOG mice is an exciting step forward in the development of humanized mice for the study of T cell responses to tumor-associated antigens (TAAs). The expression of Class I and II HLA should ultimately provide the chance to preclinically evaluate tumor vaccination strategies in which both the generation of HLA restricted tumor-specific T cells and their therapeutic effect on tumor growth can be determined.

Large animal models, like non-human primates, allow for the study of the immune response but not cancer

While studies in rodent models offer the advantages of testing the potency and therapeutic efficacy of cancer immunotherapies or vaccines, they cannot predict efficacy when doses are scaled-up for human patients, particularly when dealing with self-tumor antigens and immune tolerance. Human and mouse immune systems show discrepancies in both innate and adaptive immunity, including in leukocyte subset balance, defensins, Toll-like receptors, inducible NO synthase, NK inhibitory receptors, FcR, Ig subsets, some B cell and T cell signaling pathway components, γδ T cells, cytokines and cytokine receptors, Th1/Th2 differentiation, costimulatory molecule expression and function, antigen-presenting function of endothelial cells as well as in chemokine and chemokine receptor expression [19]. This limitation can be overcome by testing vaccination regimens in large animals with immune systems which are more similar to those in humans, such as in non-human primates (NHPs) (Fig. 1d). NHPs such as macaques are valid models to determine the safety and immunogenicity of candidate vaccines that are being developed [20]. Their immune response is similar to that of humans and so the past two decades have seen numerous immunogenicity studies use NHPs to test preclinical bacterial or viral recombinant protein-based candidate vaccines. Other studies still have tested human proteins or TAA encoding vectors [21, 22]. Although human and NHP proteins are highly homologous, the resulting immune response may not reflect outcomes in humans, since the antigen may be recognized as a non-self protein. To assess the impact of vaccination strategies in breaking immune tolerance, we have cloned rhesus ortholog TAA genes to generate genetic cancer vaccines [23]. We have also determined how important single nucleotide polymorphisms are in breaking immune tolerance to a self-antigen like HER2/neu [24].

NHPs entail two important limitations: (1) cost: in general, only pharmaceutical companies or large research institutes can afford the expensive studies associated with these animals; (2) lack of efficacy: NHPs allow the immunologic assessment of a cancer vaccine to be carried out in healthy individuals but cannot help in determining its therapeutic efficacy and impact on tumor-induced immune suppression, since spontaneous cancer is very rare even in large NHP colonies. Therefore, while they are a relevant model for the scale-up of safety and immunology studies, NHPs do not fully recapitulate cancer disease conditions and immune system impact.

Naturally occurring tumors in companion animals: an undervalued resource

Similarities between human and pet tumors

The study of spontaneous tumors in companion animals is gaining momentum (Fig. 1e). Cancer in pets is a naturally occurring disease and as common as in humans [25]. It is a leading cause of death in dogs and cats, especially now that they are living long enough to develop the disease. Several organizations are involved in advancing the knowledge of cancer in pets. These specialists include teams in veterinary surgery, radiation oncology, medical oncology and clinician/researchers (AVBC, Australia/New Zealand-http://www.avbc.asn.au/; ACVS-https://www.acvs.org/; ACVR-http://www.acvr.org/; American (www.acvim.org) and European (www.ecvim-ca.org) Colleges of Veterinary Internal Medicine, Veterinary Cancer Society, VCS).

The higher risk associated with age and behavior and, in some cases, the similar antigen expression patterns of many cancers in domestic animals mirror human disease, making the treatment of pet tumors with novel therapies critical to advancing human patient care [26]. Pet tumors grow in an intact immune system, allowing the complex interactions between the tumor and the immune system to develop naturally. This makes tumors susceptible to the selective pressure of spontaneous immunity and leads to the intratumoral heterogeneity and genetic instability [27, 28] that faithfully reproduce human cancers.

Dogs are the most frequently studied, and as they live in close proximity with humans, dogs are afflicted by the same cancers [29–31], and provide an opportunity to address not only genetic risk of disease, but also nutritional and environmental factors that are crucial for human tumor development [32, 33]. Spontaneous cancers in dogs grow over long periods of time in a syngeneic microenvironment shaped by the natural evolution of the tumor mass, and often give rise to recurrences and metastases, mimicking the progression of human tumors better than other preclinical models [34]. The existence of different breeds of dogs is analogous to the genetic diversity of the human population.

The recent release of the entire canine genome sequence has proven that its homology with the human genome is stronger than that between mouse and human genome [35]. Comparative gene expression studies have revealed close correspondence, in terms of tumor genetics and molecular

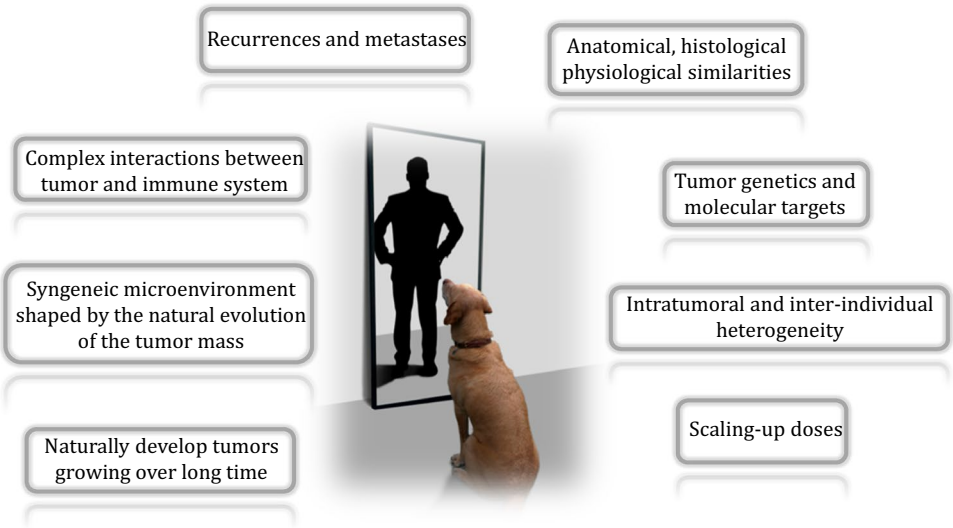

Fig. 2 Mirroring the human reality: the importance of the canine avatar. The many similarities between canine and human cancers make naturally occurring tumors in companion animals a mirror of the human clinical condition. Spontaneous tumors in pet animals grow over long periods of time in a syngeneic microenvironment, experience complex interactions between the tumor and the immune system and retrace the natural evolution of human tumors (giving rise to recurrences and metastases). They therefore mimic the progression of human disease better than other preclinical models. The significant anatomical, histological and physiologic similarities between pet and human cancers, in terms of tumor onset, progression and treatment, as well as the identification of common tumor genetics and molecular targets, effectively increase the translational power of canine models to accelerate the development of new antitumoral therapies in human patients. Canine tumors realistically mirror the complexity of human cancers thanks to their intratumoral genetic instability and patient heterogeneity. Canine cancer models are of great translational value in the role of avatars of human tumor behavior and therapy response

targets, between canine and human tumors [36–38], thus supporting the use of canine cancer models as a mirror for what occurs in human cancer biology. The finding of common driver oncogenes and deregulated cancer pathways in dogs and humans means canine tumors act with similar biological behavior and provide a similar response to conventional therapies [25, 26]. As a result, spontaneous cancer in dogs may reproduce the biological and clinical complexity of human tumors in a manner that is not possible for other preclinical models [6] (Fig. 2).

The translational power of naturally occurring pet tumors

The similarities between pets and humans with respect to anatomy, physiology, tumor onset and progression make canine tumor models a valuable tool for identifying new cancer-associated genes and for enhancing our understanding of tumor molecular biology. In addition, dog models will allow for the evaluation and development of novel diagnostic, prognostic and therapeutic applications that can benefit both dog and human cancer patients [6]. Cancer treatment in dogs includes many of the same drugs used in humans, predominantly via off-label drug use [39, 40]. Dogs and humans often have the same responses to therapies, and therefore, studies in dogs may provide useful information about drug toxicity and the mechanisms underlying the resistance of human patients to chemotherapy. There is no "gold standard" treatment for many canine cancers and so it is possible to evaluate new agents as first-line therapies, in combination with other treatments, or as adjunctive therapies in an expedited and efficient manner [41]. Moreover, as several cancer-associated genetic alterations that influence cancer progression in humans have been identified in canine cancer [36, 42, 43], testing new targeted therapies for cancer treatment holds great translational value for proof-of-concept and proof-of-target efficacy.

Naturally occurring tumors develop over long periods and constantly interact with the syngeneic immune system of the canine patients, shaping the immune response and the immune environment and mimicking the natural cancer immunoediting of human patients. Therefore, evaluating the efficacy of novel immunotherapies in animal patients may be strongly predictive of their clinical efficacy.

The rationale for evaluating therapeutics in domestic animals before carrying out in human studies is clear: (1) They provide a unique opportunity to evaluate both the safety and activity of a novel drug and have high translational value due to the similarities between canine and human tumors; (2) they offer a valuable means to assess treatment options which can be rapidly translated to human clinical use. These data are time-consuming, labor intensive and difficult to complete in conventional preclinical models or human clinical trials alone [44]. Furthermore, the inclusion of dogs from different breeds provides cross-sectional value that is often higher than in studies of inbred laboratory animals [26, 45]. The heterogeneity and complexity of cancer in the pet dog population also offer great opportunities for the development and optimization of molecularly guided analyses, which characterize personalized medicine [46].

Whereas there are strict regulations for human clinical trials, there are fewer restrictions for phase I/II/III trials in domestic animals with informed consent being a necessary regulation [44]. The reduced regulatory guidelines and the naturally shorter life spans of canine patients allow for the rapid development and completion of clinical trials that can assess outcomes in a 6- to 18-month window. This is impossible in human cancer trials [47]. The value of comparative oncology trials has been increasingly recognized as a potent translational means to assess the safety and efficacy of a study [48]. Veterinary clinical studies are becoming an "avatar" for the human setting, providing an easier way to study human cancer and innovative strategies to battle it. A number of translational contributions have originated from studies in pets (reviewed in [26]) which include the use of several targeted kinase inhibitors [49], L-MTP-PE for the treatment of osteosarcoma [50] and the first DNA vaccine to be approved by the United States Department of Agriculture (USDA) for the treatment of melanoma, ONCEPT (see later).

The Comparative Oncology Program and the LUPA Project: a way to provide new therapeutic opportunities for both pets and humans

The surveillance of cancer in pets has become more frequent and an even more important challenge for the veterinary field in recent years. Pets are also members of the family for many people, thus motivating pet owners ("pet parents") to seek out advanced therapies for the management of cancer in their companion animals. The National Cancer Institute's Center for Cancer Research (CCR) of the USA established the Comparative Oncology Program (COP) in 2003 to help advance an understanding of the biology of cancer and to ascertain the benefit of novel treatments for humans by evaluating the response of these treatments in naturally occurring cancers in pet animals—primarily cats and dogs.

The COP (https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home) designs and organizes clinical trials in collaboration with academic veterinary institutions across the USA. Pets may receive treatment under board-certified veterinary oncologists. Participation within these trials does require travel to specific veterinary academic centers, which is not always possible for even the most dedicated pet parent. The website, www.vetcancertrials.org,

was developed and is maintained by the University of Missouri-Columbia Veterinary Medical Teaching Hospital and is designed for use by everyone involved in the treatment of pet animals with cancer, including pet owners, general practice veterinarians, board-certified oncologists and other specialist veterinarians. Information is provided on clinical trials from both private practices and academically based veterinarians to favor their rapid completion while providing progressive treatment options for pets with cancer. There are almost 90 trials listed currently, and more trials are added every month. Some trials are fully funded, while others require financial outlay. The site is an invaluable asset in the quest for progressive treatment options, is supported by the VCS, and was originally developed by the Veterinary Cooperative Oncology Group (VCOG). VCS is a group of board-certified veterinary oncologists and associated specialists assembled to facilitate high-quality veterinary oncology. VCOG also promotes collaborative investigations.

A European initiative to use dogs as models for the study of common complex diseases in humans, including cancer, was formed and funded in 2008 by the European Commission (http://www.eurolupa.eu). The LUPA project [51] was named after the female wolf, which fed the twin founders of Rome, Romulus and Remus, and was initiated to highlight how humans may benefit from genetic studies on dogs. The project consists of 22 collaborating veterinary faculties and research centers which target five overlapping disease categories including cancer.

An example of their collective effort is the fact that SNP genotypes, collected as part of the project, are stored in a central database. LUPA partners have identified loci associated with susceptibility to several complex disorders and more importantly have improved the dialog between veterinary clinicians and geneticists throughout Europe and the rest of the world [52–54].

The most recent translational contribution can be found in the drug Toceranib (Palladia) from Pfizer Animal Health, now Zoetis. Like the human cancer drug Sutent, Palladia was born at Sugen, a company that was acquired by Pharmacia, which in turn was bought by Pfizer. The drug is a multi-kinase inhibitor that targets several receptor tyrosine kinases and is FDA approved for the treatment of Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement in dogs [55].

Cancer immunotherapy in dogs

A limitation of cancer immunotherapy in dogs has been the relatively poor knowledge and understanding of the canine immune system, mainly due to a lack of reagents, such as antibodies which are able to identify specific subpopulations. Such tools have recently become available, and several studies have identified immune cells which play crucial roles in canine cancer immunology, such as T regulatory cells [56, 57], myeloid-derived suppressor cells [58], NK cells [59] and tumor macrophages [60]. This increased knowledge has further solidified the position of dogs as a translational model for cancer immunotherapies. The following paragraphs summarize the most relevant efforts.

Lymphosarcoma

An example of the translational relevance of canine cancer is non-Hodgkin lymphoma (NHL), the most common canine malignancy, which accounts for up to 24 % of all reported neoplasms. The majority of canine NHL (60–80 %) arises from malignant B cells, as is the case in humans [61]. This disease has shown positive association with exposure to herbicides, chemicals and with living in highly polluted areas [62–64]. Significant association between the distributions of human and canine NHL and environmental factors such as waste incinerators, polluted sites and radioactive waste has been found in a French study [65].

Malignant lymphosarcoma (LSA) is the most common NHL in dogs. The median age of occurrence is around 7 years [66, 67]. The two standard-of-care treatments for canine B-2 lineage NHL are chemotherapy regimens: cyclophosphamide, vincristine and prednisolone (COP), and cyclophosphamide, vincristine, doxorubicin and prednisone (CHOP). These result in temporary remission in approximately 85 % of patients, but are rarely curative, as the two-year survival rate is lower than 20 % [68]. A shorter but dose-intense CHOP chemotherapy schedule results in a median survival time of approximately 27 weeks [69]. Combination protocols have generally been in favor; however, single agent protocols have provided extended survival and should be considered [66, 70].

Its high frequency in the pet population and intense medical need have made canine LSA a suitable model for innovative therapies. Recent reports have shown that canine LSA is treatable with experimental immunotherapy, such as adoptive cell therapy [71], tumor RNA-loaded, CD40-activated B cell [72] and autologous heat shock protein complexes [73], in addition to standard chemotherapy. These studies have reported significant delays in tumor progression and occasionally complete remission, thus demonstrating the susceptibility of this tumor type to immunotherapy. However, these personalized cell therapy agents are cumbersome and generally very expensive. For these reasons, alternative technologies which combine lower manufacturing costs and more standardized production processes are needed. Gene-based vaccines are a promising avenue. Research by some of the authors (LA, JAI) shows that a

genetic vaccine which targets dog telomerase reverse transcriptase was immunogenic in almost all treated animals and, most importantly, in a double-armed trial had significant therapeutic impact on canine LSA [74, 75].

Monoclonal canine antibodies for the treatment of canine LSA are also attracting interest. Rituximab, a chimeric monoclonal antibody, has previously been evaluated for binding against canine B cells in NHL, but no in vivo depletion was identified [76].

Aratana (www.aratana.com) is a US company that is actively involved in this technology. They are developing AT-005 for T cell lymphoma. AT-005 is a canine version of Campath, which is a drug developed for human targeting CD52. Similarly, AT-004 mAb provides dogs with targeted immunotherapy against the cell-surface antigen CD20, which is expressed on canine lymphoma B cells. AT-004 depletes malignant B cells.

Genetic vaccines and canine mAb may therefore be convenient and uniquely targeted products which can complement standard LSA treatment.

Melanoma

Malignant melanoma (MM) is a spontaneous tumor in dogs and is responsible for 7 % of all malignant tumors [77]. It is the most common malignant neoplasm of the oral cavity [78], while other less commonly affected sites are the lips (23 %), skin (11 %) and digits (8 %) [77]. Generally, MM is detected at an advanced stage when tumor resection is rarely curative and metastases are already present. Clinical biological malignancy is mainly attributed to oral melanomas as they are almost all malignant and display a metastatic rate of up to 80 % to regional lymph nodes and other organs, including the lungs, and thus mimic the clinical evolution of human disease [77, 79].

Although they differ in frequency and severity [77, 80], canine and human melanomas share many similarities, including the same anatomical sites, similar histopathology and common architectural features [81]. Several studies have focused on the evaluation of tumor genetics and canine MM molecular targets, leading to the identification of common hotspot somatic mutations in dogs and humans, suggesting that common pathways contribute to the progression of the disease in both species [82, 83]. A similar differential gene expression pattern in the MAPK "mitogenic" pathway and in the PI3K/Akt "survival" pathway, primarily involved in human MM tumorigenicity, has been identified in canine MM [83], laying the foundation for more rational therapeutic comparative studies. Furthermore, the absence of the BRAF somatic mutation in canine MM, which mostly develops in non-UV-exposed sites, is paralleled in human non-UV-linked MM which also harbors wild-type BRAF. This highlights the relevance of the canine MM model in the study of human homologous, non-UV-linked MM subtypes and the identification of new therapeutic targets for wild-type BRAF patients.

The conventional management of canine MM, and especially of the most aggressive oral type, is often as disappointing as it is in humans. Traditional treatment for canine MM involves surgery, radiotherapy and chemotherapy and is efficient in controlling the tumor locally in up to 75 % of animals, whether used alone or in combination. However, the 1-year survival rate does not exceed 30 %, because of metastasis [84–86].

Several comparative studies of novel immunotherapy protocols have been performed in dogs affected by MM, and promising results have been achieved [87–91]. These efforts led to the development of the first USDA-approved antitumor vaccine; ONCEPT (Merial), a xenogeneic DNA vaccine targeting tyrosinase which can extend survival in dogs with locally controlled stage II–III oral MM. This vaccine is widely used and gives encouraging results [92, 93]. Nevertheless, a recent retrospective study conducted on a limited number of dogs has questioned its efficacy [94].

ONCEPT approval spurred the development and evaluation of other vaccines. Mayayo et al. [95] were the first to investigate the expression of chondroitin sulfate proteoglycan (CSPG) 4 in canine MM. It is an early cell-surface progression marker which is highly expressed in about 80 % of human MM where it regulates tumor cell proliferation, migration and invasion [96]. Mayayo et al. [95] found CSPG4 expression in about 60 % of canine MM and labeled it as a new marker for canine MM diagnosis and a promising immunotherapy target. Two of this review's authors (FC, FR) have now tested a xenogeneic DNA vaccine against this molecule in client-owned dogs with surgically resected stage II–III CSPG4-positive, spontaneous oral MM. The disease-free interval and overall survival of vaccinated dogs were significantly longer than in the controls: 477 versus 180 and 653 versus 220 days, respectively [97].

Mammary carcinoma

Canine mammary tumors (CMT) share many characteristics with human breast cancer, including histological appearance, biological behavior, hormone dependence, frequent oncogene HER-2/neu activation [98, 99] and response to conventional treatments. Human and dog gene expression data, from both tumor and normal mammary samples, show that a significant number of shared genes are deregulated in the tumors as compared to their normal counterparts. Pathway analysis of gene expression data reveals a high degree of similarity in the perturbation of many cancer-related pathways. The transcriptional relationships between various human breast cancer gene signatures

are mostly maintained in the canine sequences, suggesting that CMT may work as a translational model for human disease [100]. Similarly, feline mammary tumors (FMT) show protein and gene expression profiles that are comparable to human cancers [101, 102].

Standard therapies include surgical extirpation of the gland (dog) versus radical bilateral mastectomies (cat) followed with chemotherapy. No standard chemotherapy protocol is known to be effective, and continued research is being pursued to offset metastasis which leads to euthanasia. Mammary tumors are associated with a high risk of metastatic disease, especially in cats, and several studies indicate that HER-2/neu expression is similar in human breast carcinoma [103]. For all these reasons, CMT and FMT are ideal preclinical models with which to evaluate HER-2/neu immunotherapy. A genetic vaccine based on a combination of adenovirus and DNA electroporation has been shown to be immunogenic in dogs [104], and some authors (LA, JAI) are currently testing its antitumor efficacy in FMT and CMT.

Osteosarcoma

Osteosarcoma (OSA) is a primary bone tumor that most commonly affects the medullary canal of long bone metaphyses. It is similar in humans [105], and it is estimated that over 8,000 dogs per year will be diagnosed with OSA in the USA. Common sites are the distal radius, proximal humerus, distal femur and proximal tibia, but finding OSA at other sites is not unusual. Most affected patients suffer lameness and/or the development of a firm mass at the primary site. Of the primary bone tumors reported to occur in dogs, OSA is the most common and accounts for more than 80 % of all canine primary bone cancers. The average age of canine sufferers is 7 years, but can range from 6 months to more than 12 years. Amputation alleviates pain and decreases the risk of pathologic fracture. Without adjuvant therapy, amputation must be considered a pain-palliative procedure only, as it does not significantly increase survival time, but improves the quality of life. Patients usually succumb to lung metastasis.

Amputation and systemic chemotherapy is the current treatment of choice for canine appendicular OSA. Postoperative systemic chemotherapy is currently used to suppress the development of metastatic disease, but is ineffective. Two meta-analysis studies have recently been published and confirm the roles of serum alkaline phosphatase (SALP) and proximal humeral location as negative prognostic factors and gave a median survival time of 256 days [106, 107]. Many patients are poor candidates for amputation, due to mitigating factors such as severe degenerative joint disease, obesity and multiple tumor sites. Some owners resist the amputation of their pets' limbs because they are reluctant to subject them to this radical procedure.

OSA is a suitable cancer for immunotherapy due to the frequency of metastatic disease despite local control. A common feature of OSA is the expression of the TAAs, HER2/neu and/or CSPG4. An autologous tumor cell vaccine, genetically engineered to express hGM-CSF [108], was once suggested to induce an immune response and give a therapeutic outcome. More recently, Advaxis has developed technology that uses attenuated, live *Listeria* as a vector to deliver a tumor-associated antigen in order to activate the patient's immune system. This protocol has been explored in OSA-affected humans and dogs (www.advaxis.com). *Listeria monocytogenes* strains have been engineered to induce an innate immune response and to express tumor-associated antigens which induce tumor-specific T cell-mediated immunity. In addition, tumor antigens have been fused to virulence factor listeriolysin (LLO) in the *Listeria* bacterium. The combination of the tumor antigen and LLO generates a strong immune response, which attacks the cancer. ADXS-cHER2 is an immunotherapy treatment based on this technology that targets the HER2 oncogene. An ongoing Phase I trial at the University of Pennsylvania is treating naturally occurring OSA-suffering pet dogs with ADXS-cHER2, after their standard-of-care treatment, and shows significantly prolonged overall survival over dogs that received the standard-of-care treatment without ADXS-cHER2 (Advaxis press release). On this basis, Advaxis announced that it intends to initiate a clinical program of ADXS-cHER2 for the treatment of pediatric osteosarcoma. In addition, Advaxis signed a global licensing agreement with Aratana Therapeutics, Inc. for ADXS-cHER2 for the treatment of osteosarcoma in dogs. Two authors (LA, JI) have also been evaluating HER2 immunotherapy against canine osteosarcoma using the prime/boost technology with electrogenetransfer. Patient accrual is ongoing.

Conclusions

Our increasing knowledge of cancer biology and its mechanisms and tumors' complex interaction with microenvironments and immune systems is leading to a new vision of translational oncology. Investigations into new drugs and vaccines, their combinations and the assessment of biomarkers and responding histologies can now rely on a variety of animal models which are much closer to human diseases.

Comparative oncology has undergone tremendous growth over the last 30 years, and the continuation of this collaborative effort can only hasten important discoveries as to the mechanics of cancer and therapeutic intervention,

which will bring benefits to both dogs and humans alike. Clinical trial funding, ever the challenge, will become easier to justify with the use of naturally occurring cancer models. Indeed, the treatment of canine patients today could be of immense help for their owners tomorrow. This is the main motivation that, in recent years, has moved veterinarians, pathologists, researchers, clinicians and pet owners themselves to collaborate and combine knowledge and efforts. The final aim is to transform the concept of comparative oncology into a more efficient and concrete tool for translational medicine.

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Conflict of interest The authors declare no conflict of interest.

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