UNIVERSITÀ DEGLI STUDI DI TORINO

Riccardo F, Aurisicchio L, Impellizeri JA, Cavallo F.

The importance of comparative oncology in translational medicine.

In Cancer Immunol Immunother. 2015 Feb;64(2):137-48

DOI: 10.1007/s00262-014-1645-5

The importance of comparative oncology in translational medicine

Federica Riccardo[1], Luigi Aurisicchio[2], Joseph Impellizeri[3], Federica Cavallo[1]

[1]Department of Molecular Biotechnologies and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy

[2]Takis s.r.l., Rome, Italy

[3]DVM, DACVIM, MRCVS, Veterinary Oncology Services, New York, USA

Corresponding author:
Federica Cavallo
University of Torino
Molecular Biotechnology Center
Via Nizza, 52
10126, Torino
Italy
e-mail: federica.cavallo@unito.it

Abstract

Human cancer is complex to such an extent that *in vivo* pre-clinical 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 is a leading cause of death in companion animals now that more pets are living long enough to develop the disease, while more owners are seeking advanced and novel therapies for them 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.

Précis

Cancer therapeutics has been limited to translational murine models for decades. Naturally occurring pet cancer models may provide more accurate treatment assessment and expedite approval thus furnishing potential benefit to all species battling cancer.

Keywords

Cancer models; canine tumors; tumor associated antigens; immunotherapy

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 (Figure 1).

For many years, the study of cancer cell lines has been the elective experimental model (Figure 1a) and 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 the testing therapeutic drugs (1, 2). Nevertheless, heterotypic interactions between the tumor and the multiple distinct cell types of 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) (Figure 1b). These transplantable models can be standardized and provide reproducible data, but are 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 the 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) (Figure 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) intra-tumor 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 that might better reflect a clinical response (9), being of help for the choice of the most appropriate drug to be used for that patient. This represents an important step forward personalized medicine but not without pitfalls. The mouse one little by little replaces the implanted patient stroma, while the mouse immune system is not functional and both these aspects could affect the translational value of the results. Moreover the entire strategy of implantation of the patient tumor in mice (not always successful) and of drugs testing may take longer becoming a race against the 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 the interaction between the tumor 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) (Figure 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 different 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 throughout the tissue(s) where the promoter is activated and for the mouse entire life is (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 pre-clinical 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) (Figure 1b). However, most existing humanized mouse models cannot develop human innate immune cells, including myeloid cells and NK cells. Two mouse strains, MITRG and MISTRG, have been recently described in which four human genes which encode cytokines and that are 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 advance in 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 pre-clinically evaluate tumor vaccination strategies in which both the generation of MHC 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 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, 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 nonhuman primates (NHPs) (Figure 1d). NHPs such as macaques are valid models to determine the safety and immunogenicity of candidate vaccines that are being developed (27). Their immune response is similar to that of humans, and within the past two decades numerous immunogenicity studies have used NHPs to test pre-clinical candidate vaccines consisting of bacterial or viral recombinant proteins. Other

studies have tested human proteins or TAA encoding vectors (28, 29). Although human and NHPs proteins share high homology, 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 a vaccination strategy in breaking immune tolerance, we have cloned rhesus orthologue TAA genes to generate genetic cancer vaccines (20). We have also determined how important single nucleotide polymorphisms are in breaking immune tolerance to a self-antigen like HER2/neu (21).

NHPs carry with them 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 be useful 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 (Figure 1e). Cancer in pets is a naturally occurring disease and as common as in humans (22). 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 cure (23). Pet tumors develop in an intact immune system, allowing the complex interactions between the tumor and the immune system to occur. This makes tumors susceptible to the selective

pressure of spontaneous immunity and leads to the intratumoral heterogeneity and genetic instability (24, 25) that faithfully reproduce human cancers.

Dogs are the most studied. Living in close proximity with humans, dogs are afflicted by the same cancers (26-28), and provide an opportunity to address not only genetic risk for disease, but also nutritional and environmental factors that are crucial for human tumor development (29, 30). 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 (31). The existence of different breeds in the dog population means that the heterogeneity in patients with the same disease reflects the diversity of human cancers.

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 (32). Comparative gene expression studies have revealed close correspondence, in terms of tumor genetics and molecular targets, between canine and human tumors (33-35), 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 biologic behavior and provide a similar response to conventional therapies (22, 23). 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) (Figure 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 (46, 47). 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 (36). Moreover, as several cancer associated genetic alterations that influence cancer progression in humans have been identified in canine cancer (33, 37, 38), 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 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 (39). Furthermore, the inclusion of dogs from different breeds provides cross-sectional value that is often higher than in studies of inbred laboratory animals (23, 40). The heterogeneity and complexity of cancer in the pet dog population also offers great opportunities for the development and optimization of molecularly guided analysis, which characterizes personalized medicine (41).

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 (39). 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 6-18 month window. This is impossible in human cancer trials (42). The value of comparative oncology trials has been increasingly recognized as a potent translational means to assess the safety, efficacy, suitable human dosage and clinically relevant endpoints of a study (43). 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 (23)) which include the use of several targeted kinase inhibitors (44), L-MTP-PE for the treatment of osteosarcoma (45) 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 intense and an important challenge in 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 United States 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 United States. 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 for clinical trials from both private practices and academically based veterinarians to favor the rapid completion of clinical trials 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 a model 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 (46) 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 dialogue between veterinary clinicians and geneticists throughout Europe and the rest of the world (47-49).

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 (50).

Cancer immunotherapy in dogs

A limitation of cancer immunotherapy in dogs has been the relatively poor knowledge and understanding of 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 (51, 52), myeloid derived suppressor cells (53), NK cells (54) and tumor macrophages (55). 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%) arise from malignant B cells, as is the case in humans (56). This disease has shown a positive association with exposure to herbicides, chemicals and with living in highly polluted areas (57-59). Significant association between the distributions of human, canine NHL and environmental factors such as waste incinerators, polluted sites and radioactive waste was found in a French study (60).

Malignant lymphosarcoma (LSA) is the most common NHL in dogs. The median age of occurrence is around 7 years (61, 62). The two standard-of-care treatments for canine B-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% (63). A shorter but dose-intense CHOP chemotherapy schedule results in a median survival time of approximately 27 weeks (64). Combination protocols have generally been in favor, however single agent protocols have provided extended survival and should be considered (61, 65).

Due to its high frequency in the pet population and an intense medical need, canine LSA is a suitable model for innovative therapies. Recent reports have shown that canine LSA is treatable with experimental immunotherapy, such as adoptive cell therapy (66), tumor RNA-loaded, CD40-activated B cell (67) and autologous Heat Shock protein complexes (68), 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) show 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 a significant therapeutic impact on canine LSA (69, 70).

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 (71).

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 a convenient and uniquely targeted product which can complement standard LSA treatment.

Melanoma

Malignant melanoma (MM) is a spontaneous tumor in dogs which makes up 7% of all malignant tumors (72). It is the most common malignant neoplasm of the oral cavity (73), while other less commonly affected sites are the lips (23%), skin (11%) and digits (8%) (72). 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 (72, 74).

Although they differ in frequency and severity (84, 87), canine and human melanomas share many similarities, including the same anatomical sites, similar histopathology and common architectural features (88). 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 (75, 76). A similar differential gene expression pattern in the MAPK “mitogenic” pathway and in the PI3K/Akt “survival” pathway, primarily involved in human MM tumorigenicity, have been identified in canine MM (76), laying the foundation for more rational therapeutic comparative studies. While 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 denotes the relevance of the canine MM model to 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 (77-79).

Several comparative studies of novel immunotherapy therapy protocols have been performed in dogs affected by MM and promising results have been achieved (80-84). These efforts led to the development of the first USDA-approved anti-tumor vaccine: the 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 (85,

86). Nevertheless, a recent retrospective study conducted on a limited number of dogs has questioned its efficacy (87).

ONCEPT approval spurred the development and evaluation of other vaccines. Mayayo et al. were the first to investigate the expression of chondroitin sulfate proteoglycan (CSPG)4 in canine MM (88). 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 (89). Mayayo and coworkers found CSPG4 expression in about 60% of canine MM (88), 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 as compared to those of controls, being 477 vs 180 and 653 vs 220 days, respectively (90).

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 (91, 92) 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 different gene signatures of human breast cancer are mostly maintained in the canine sequences, suggesting CMT as translational model for human disease (107). Similarly, feline mammary tumors (FMT) show protein and gene expression profiles that are comparable to human cancers (108, 109).

Standard therapies include surgical extirpation of the gland (dog) vs. radical bilateral mastectomies (cat) followed with chemotherapy. No standard chemotherapy protocol has been reported 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 (93). 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 (94) 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 (95) and it is estimated that over 8,000 dogs per year will be diagnosed with OSA in the United States. 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 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 confirmed serum alkaline phosphatase (SALP) and proximal humeral location as negative prognostic factors and gave a median survival time of 256 days (96, 97). 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 targeting with 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 (98), 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, with patient accrual ongoing.

Conclusions

Our increasing knowledge of cancer biology, its mechanisms and tumors' complex interaction with microenvironments and immune systems are 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 in the past 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 point that, in recent years, has moved veterinarians, pathologists, researchers, clinicians and pet owners themselves to collaborate and combine knowledge and effort. The final aim is to transform the concept of comparative oncology into a more efficient and concrete tool for translational medicine.

Acknowledgements

This work was supported by grants from the Italian Association for Cancer Research (IG 5377), the University of Turin and Fondazione Ricerca Molinette Onlus. We thank Dr. Dale Lawson for his revision and editing of the manuscript. We thank Apunto 3D Visuals (www.apunto.it) for its contribution to the creation of the figures.

Conflict of interest

The authors declare no conflict of interest.

References

1. Vargo-Gogola T, Rosen JM (2007) Modelling breast cancer: one size does not fit all. Nat Rev Cancer. 7: 659-72. doi: 10.1038/nrc2193
2. Kao J, Salari K, Bocanegra M, Choi YL, Girard L, Gandhi J et al. (2009) Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. PLoS One. 4: e6146. doi: 10.1371/journal.pone.0006146
3. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell. 144: 646-74. doi: 10.1016/j.cell.2011.02.013
4. Nelson CM, Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol. 22: 287-309. doi: 10.1146/annurev.cellbio.22.010305.104315
5. Dranoff G (2012) Experimental mouse tumour models: what can be learnt about human cancer immunology? Nat Rev Immunol. 12: 61-6. doi: 10.1038/nri3129
6. Pinho SS, Carvalho S, Cabral J, Reis CA, Gartner F (2012) Canine tumors: a spontaneous animal model of human carcinogenesis. Transl Res. 159: 165-72. doi: 10.1016/j.trsl.2011.11.005
7. Lollini PL, Cavallo F, Nanni P, Forni G (2006) Vaccines for tumour prevention. Nat Rev Cancer. 6: 204-16. doi: 10.1038/nrc1815
8. Bibby MC (2004) Orthotopic models of cancer for preclinical drug evaluation: advantages and disadvantages. Eur J Cancer. 40: 852-7. doi: 10.1016/j.ejca.2003.11.021 S0959804903010293 [pii]
9. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goere D, Mariani P et al. (2012) Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 18: 5314-28. doi: 10.1158/1078-0432.CCR-12-0372
10. Couzin-Frankel J (2014) The littlest patient. Science. 346: 24-7. doi: 10.1126/science.346.6205.24
11. Singh M, Ferrara N (2012) Modeling and predicting clinical efficacy for drugs targeting the tumor milieu. Nat Biotechnol. 30: 648-57. doi: 10.1038/nbt.2286
12. Berns A, Barbacid M (2013) Mouse models of cancer. Mol Oncol. 7: 143-5. doi: 10.1016/j.molonc.2013.02.014
13. Jacob J, Radkevich O, Forni G, Zielinski J, Shim D, Jones RF, Wei WZ (2006) Activity of DNA vaccines encoding self or heterologous Her-2/neu in Her-2 or neu transgenic mice. Cell Immunol. 240: 96-106. doi: 10.1016/j.cellimm.2006.07.002
14. Lollini PL, Cavallo F, De Giovanni C, Nanni P (2013) Preclinical vaccines against mammary carcinoma. Expert Rev Vaccines. 12: 1449-63. doi: 10.1586/14760584.2013.845530
15. Jonkers J, Berns A (2002) Conditional mouse models of sporadic cancer. Nat Rev Cancer. 2: 251-65. doi: 10.1038/nrc777
16. Bashir T, Cloninger C, Artinian N, Anderson L, Bernath A, Holmes B et al. (2012) Conditional astroglial Rictor overexpression induces malignant glioma in mice. PLoS One. 7: e47741. doi: 10.1371/journal.pone.0047741 PONE-D-12-21223 [pii]
17. Zhu C, Luong R, Zhuo M, Johnson DT, McKenney JK, Cunha GR, Sun Z (2011) Conditional expression of the androgen receptor induces oncogenic transformation of the mouse prostate. J Biol Chem. 286: 33478-88. doi: M111.269894 [pii] 10.1074/jbc.M111.269894

18. Rongvaux A, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL et al. (2014) Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 32: 364-72. doi: 10.1038/nbt.2858
19. Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. J Immunol. 172: 2731-8.
20. Aurisicchio L, Mennuni C, Giannetti P, Calvaruso F, Nuzzo M, Cipriani B et al. (2007) Immunogenicity and safety of a DNA prime/adenovirus boost vaccine against rhesus CEA in nonhuman primates. Int J Cancer. 120: 2290-300. doi: 10.1002/ijc.22555
21. Fattori E, Aurisicchio L, Zampaglione I, Arcuri M, Cappelletti M, Cipriani B et al. (2009) ErbB2 genetic cancer vaccine in nonhuman primates: relevance of single nucleotide polymorphisms. Hum Gene Ther. 20: 253-65. doi: 10.1089/hum.2008.153
22. Vail DM, MacEwen EG (2000) Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest. 18: 781-92.
23. Paoloni M, Khanna C (2008) Translation of new cancer treatments from pet dogs to humans. Nat Rev Cancer. 8: 147-56. doi: 10.1038/nrc2273
24. Dickinson PJ, LeCouteur RA, Higgins RJ, Bringas JR, Larson RF, Yamashita Y et al. (2010) Canine spontaneous glioma: a translational model system for convection-enhanced delivery. Neuro Oncol. 12: 928-40. doi: 10.1093/neuonc/noq046
25. Pena L, De Andres PJ, Clemente M, Cuesta P, Perez-Alenza MD (2013) Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. Vet Pathol. 50: 94-105. doi: 10.1177/0300985812447830
26. Bukowski JA, Wartenberg D, Goldschmidt M (1998) Environmental causes for sinonasal cancers in pet dogs, and their usefulness as sentinels of indoor cancer risk. J Toxicol Environ Health A. 54: 579-91.
27. Patronek GJ, Waters DJ, Glickman LT (1997) Comparative longevity of pet dogs and humans: implications for gerontology research. J Gerontol A Biol Sci Med Sci. 52: B171-8.
28. Mukaratirwa S, Chipunza J, Chitanga S, Chimonyo M, Bhebhe E (2005) Canine cutaneous neoplasms: prevalence and influence of age, sex and site on the presence and potential malignancy of cutaneous neoplasms in dogs from Zimbabwe. J S Afr Vet Assoc. 76: 59-62.
29. Waters DJ, Wildasin K (2006) Cancer clues from pet dogs. Sci Am. 295: 94-101.
30. Tamburini BA, Trapp S, Phang TL, Schappa JT, Hunter LE, Modiano JF (2009) Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. PLoS One. 4: e5549. doi: 10.1371/journal.pone.0005549
31. Mack GS (2006) Clinical trials going to the dogs: canine program to study tumor treatment, biology. J Natl Cancer Inst. 98: 161-2. doi: 10.1093/jnci/djj061
32. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M et al. (2005) Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 438: 803-19. doi: 10.1038/nature04338
33. Mueller F, Fuchs B, Kaser-Hotz B (2007) Comparative biology of human and canine osteosarcoma. Anticancer Res. 27: 155-64.
34. Angstadt AY, Thayanithy V, Subramanian S, Modiano JF, Breen M (2012) A genome-wide approach to comparative oncology: high-resolution oligonucleotide aCGH of canine and human osteosarcoma pinpoints shared microaberrations. Cancer Genet. 205: 572-87. doi: 10.1016/j.cancergen.2012.09.005
35. Mooney M, Bond J, Monks N, Eugster E, Cherba D, Berlinski P et al. (2013) Comparative RNA-Seq and microarray analysis of gene expression changes in B-cell lymphomas of Canis familiaris. PLoS One. 8: e61088. doi: 10.1371/journal.pone.0061088

36. Gordon IK, Khanna C (2010) Modeling opportunities in comparative oncology for drug development. ILAR J. 51: 214-20.
37. Rivera P, von Euler H (2011) Molecular biological aspects on canine and human mammary tumors. Vet Pathol. 48: 132-46. doi: 10.1177/0300985810387939
38. Ranieri G, Gadaleta CD, Patruno R, Zizzo N, Daidone MG, Hansson MG et al. (2013) A model of study for human cancer: Spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. Crit Rev Oncol Hematol. 88: 187-97. doi: 10.1016/j.critrevonc.2013.03.005
39. Khanna C, London C, Vail D, Mazcko C, Hirschfeld S (2009) Guiding the optimal translation of new cancer treatments from canine to human cancer patients. Clin Cancer Res. 15: 5671-7. doi: 10.1158/1078-0432.CCR-09-0719
40. Khanna C, Lindblad-Toh K, Vail D, London C, Bergman P, Barber L et al. (2006) The dog as a cancer model. Nat Biotechnol. 24: 1065-6. doi: 10.1038/nbt0906-1065b
41. Paoloni M, Webb C, Mazcko C, Cherba D, Hendricks W, Lana S et al. (2014) Prospective Molecular Profiling of Canine Cancers Provides a Clinically Relevant Comparative Model for Evaluating Personalized Medicine (PMed) Trials. PLoS One. 9: e90028. doi: 10.1371/journal.pone.0090028
42. Paoloni MC, Khanna C (2007) Comparative oncology today. Vet Clin North Am Small Anim Pract. 37: 1023-32; v. doi: 10.1016/j.cvsm.2007.08.003
43. Rowell JL, McCarthy DO, Alvarez CE (2011) Dog models of naturally occurring cancer. Trends Mol Med. 17: 380-8. doi: 10.1016/j.molmed.2011.02.004
44. Khanna C, Gordon I (2009) Catching cancer by the tail: new perspectives on the use of kinase inhibitors. Clin Cancer Res. 15: 3645-7. doi: 1078-0432.CCR-09-0132 [pii]
10.1158/1078-0432.CCR-09-0132
45. Anderson P (2006) Liposomal muramyl tripeptide phosphatidyl ethanolamine: ifosfamide-containing chemotherapy in osteosarcoma. Future Oncol. 2: 333-43. doi: 10.2217/14796694.2.3.333
46. Lequarre AS, Andersson L, Andre C, Fredholm M, Hitte C, Leeb T et al. (2011) LUPA: a European initiative taking advantage of the canine genome architecture for unravelling complex disorders in both human and dogs. Vet J. 189: 155-9. doi: 10.1016/j.tvjl.2011.06.013
47. Vaysse A, Ratnakumar A, Derrien T, Axelsson E, Rosengren Pielberg G, Sigurdsson S et al. (2011) Identification of genomic regions associated with phenotypic variation between dog breeds using selection mapping. PLoS Genet. 7: e1002316. doi: 10.1371/journal.pgen.1002316
48. Berglund J, Nevalainen EM, Molin AM, Perloski M, Andre C, Zody MC et al. (2012) Novel origins of copy number variation in the dog genome. Genome Biol. 13: R73. doi: 10.1186/gb-2012-13-8-r73
49. Sjostrand K, Wess G, Ljungvall I, Haggstrom J, Merveille AC, Wiberg M et al. (2014) Breed Differences in Natriuretic Peptides in Healthy Dogs. J Vet Intern Med. doi: 10.1111/jvim.12310
50. London CA, Malpas PB, Wood-Follis SL, Boucher JF, Rusk AW, Rosenberg MP et al. (2009) 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: 3856-65. doi: 10.1158/1078-0432.CCR-08-1860
51. Biller BJ, Elmslie RE, Burnett RC, Avery AC, Dow SW (2007) Use of FoxP3 expression to identify regulatory T cells in healthy dogs and dogs with cancer. Vet Immunol Immunopathol. 116: 69-78. doi: S0165-2427(06)00351-5 [pii]
10.1016/j.vetimm.2006.12.002

52. Biller BJ, Guth A, Burton JH, Dow SW (2010) Decreased ratio of CD8+ T cells to regulatory T cells associated with decreased survival in dogs with osteosarcoma. J Vet Intern Med. 24: 1118-23. doi: JVIM557 [pii]
10.1111/j.1939-1676.2010.0557.x
53. Sherger M, Kisseberth W, London C, Olivo-Marston S, Papenfuss TL (2012) Identification of myeloid derived suppressor cells in the peripheral blood of tumor bearing dogs. BMC Vet Res. 8: 209. doi: 1746-6148-8-209 [pii]
10.1186/1746-6148-8-209
54. Michael HT, Ito D, McCullar V, Zhang B, Miller JS, Modiano JF (2013) Isolation and characterization of canine natural killer cells. Vet Immunol Immunopathol. 155: 211-7. doi: S0165-2427(13)00195-5 [pii]
10.1016/j.vetimm.2013.06.013
55. Guth AM, Hafeman SD, Elmslie RE, Dow SW (2013) Liposomal clodronate treatment for tumour macrophage depletion in dogs with soft-tissue sarcoma. Vet Comp Oncol. 11: 296-305. doi: 10.1111/j.1476-5829.2012.00319.x
56. Ponce F, Marchal T, Magnol JP, Turinelli V, Ledieu D, Bonnefont C et al. (2010) A morphological study of 608 cases of canine malignant lymphoma in France with a focus on comparative similarities between canine and human lymphoma morphology. Vet Pathol. 47: 414-33. doi: 10.1177/0300985810363902
57. Gavazza A, Presciuttini S, Barale R, Lubas G, Gugliucci B (2001) Association between canine malignant lymphoma, living in industrial areas, and use of chemicals by dog owners. J Vet Intern Med. 15: 190-5.
58. Hayes HM, Tarone RE, Cantor KP, Jessen CR, McCurnin DM, Richardson RC (1991) Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. J Natl Cancer Inst. 83: 1226-31.
59. Miligi L, Costantini AS, Veraldi A, Benvenuti A, Will, Vineis P (2006) Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. Ann N Y Acad Sci. 1076: 366-77. doi: 10.1196/annals.1371.036
60. Pastor M, Chalvet-Monfray K, Marchal T, Keck G, Magnol JP, Fournel-Fleury C, Ponce F (2009) Genetic and environmental risk indicators in canine non-Hodgkin's lymphomas: breed associations and geographic distribution of 608 cases diagnosed throughout France over 1 year. J Vet Intern Med. 23: 301-10. doi: 10.1111/j.1939-1676.2008.0255.x
61. Simon D, Moreno SN, Hirschberger J, Moritz A, Kohn B, Neumann S et al. (2008) Efficacy of a continuous, multiagent chemotherapeutic protocol versus a short-term single-agent protocol in dogs with lymphoma. J Am Vet Med Assoc. 232: 879-85. doi: 10.2460/javma.232.6.879
62. Gavazza AS, F; Lubas, G; Gugliucci, B; Valori, E (2009) Clinical, laboratory, diagnostic and prognostic aspects of canine lymphoma: a restrospective study. Comp Clin Pathol.
63. Marconato L, Gelain ME, Comazzi S (2013) The dog as a possible animal model for human non-Hodgkin lymphoma: a review. Hematol Oncol. 31: 1-9. doi: 10.1002/hon.2017
64. Chun R (2009) Lymphoma: which chemotherapy protocol and why? Top Companion Anim Med. 24: 157-62. doi: 10.1053/j.tcam.2009.03.003
65. Higginbotham ML, McCaw DL, Roush JK, Nietfeld JC, Wilkerson MJ, Reeds K, Burr D (2013) Intermittent single-agent doxorubicin for the treatment of canine B-cell lymphoma. J Am Anim Hosp Assoc. 49: 357-62. doi: 10.5326/JAAHA-MS-5929
66. O'Connor CM, Sheppard S, Hartline CA, Huls H, Johnson M, Palla SL et al. (2012) Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy. Sci Rep. 2: 249. doi: 10.1038/srep00249

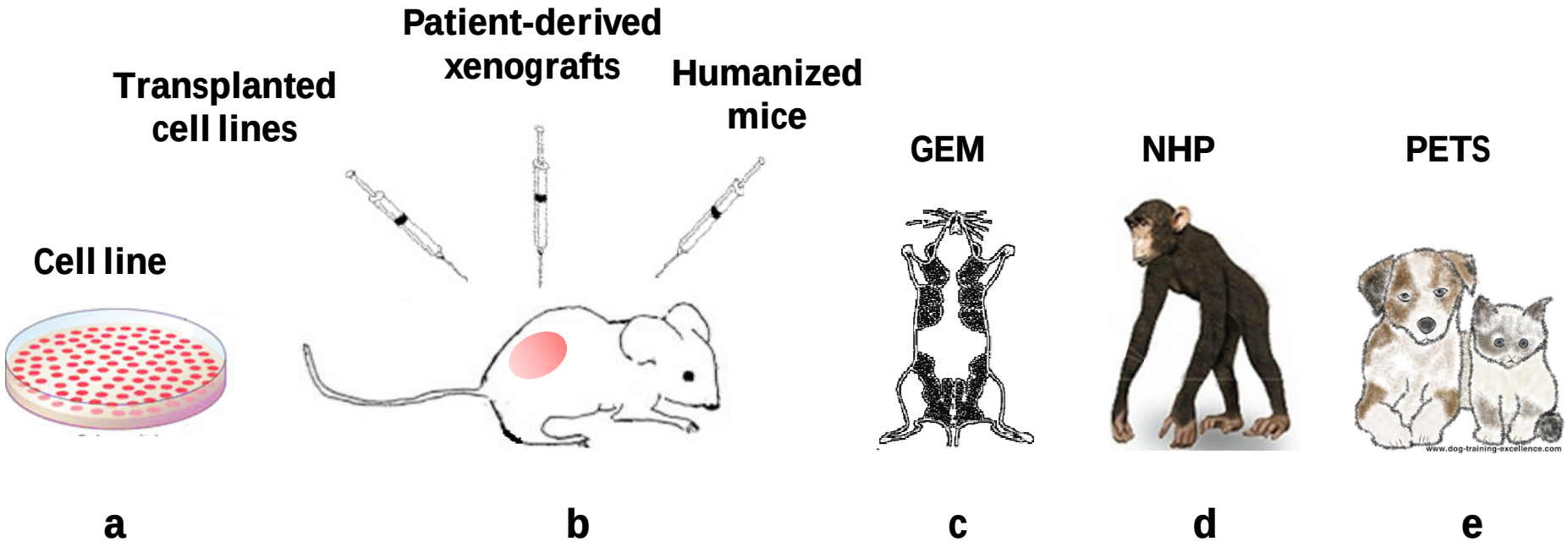
67. Sorenmo KU, Krick E, Coughlin CM, Overley B, Gregor TP, Vonderheide RH, Mason NJ (2011) CD40-activated B cell cancer vaccine improves second clinical remission and survival in privately owned dogs with non-Hodgkin's lymphoma. PLoS One. 6: e24167. doi: 10.1371/journal.pone.0024167
68. Marconato L, Frayssinet P, Rouquet N, Comazzi S, Leone VF, Laganga P et al. (2014) Randomized, placebo-controlled, double-blinded chemoimmunotherapy clinical trial in a pet dog model of diffuse large B-cell lymphoma. Clin Cancer Res. 20: 668-77. doi: 10.1158/1078-0432.CCR-13-2283
69. Peruzzi D, Gavazza A, Mesiti G, Lubas G, Scarselli E, Conforti A et al. (2010) A vaccine targeting telomerase enhances survival of dogs affected by B-cell lymphoma. Mol Ther. 18: 1559-67. doi: 10.1038/mt.2010.104
70. Gavazza A, Lubas G, Fridman A, Peruzzi D, Impellizeri JA, Luberto L et al. (2013) Safety and efficacy of a genetic vaccine targeting telomerase plus chemotherapy for the therapy of canine B-cell lymphoma. Hum Gene Ther. 24: 728-38. doi: 10.1089/hum.2013.112
71. Impellizeri JA, Howell K, McKeever KP, Crow SE (2006) The role of rituximab in the treatment of canine lymphoma: an ex vivo evaluation. Vet J. 171: 556-8. doi: 10.1016/j.tvjl.2005.03.005
72. Smith SH, Goldschmidt MH, McManus PM (2002) A comparative review of melanocytic neoplasms. Vet Pathol. 39: 651-78.
73. Boston SE, Lu X, Culp WT, Montinaro V, Romanelli G, Dudley RM et al. (2014) Efficacy of systemic adjuvant therapies administered to dogs after excision of oral malignant melanomas: 151 cases (2001-2012). J Am Vet Med Assoc. 245: 401-7. doi: 10.2460/javma.245.4.401
74. Bergman PJ (2007) Canine oral melanoma. Clin Tech Small Anim Pract. 22: 55-60. doi: 10.1053/j.ctsap.2007.03.004
75. Gillard M, Cadieu E, De Brito C, Abadie J, Vergier B, Devauchelle P et al. (2014) Naturally occurring melanomas in dogs as models for non-UV pathways of human melanomas. Pigment Cell Melanoma Res. 27: 90-102. doi: 10.1111/pcmr.12170
76. Fowles JS, Denton CL, Gustafson DL (2013) Comparative analysis of MAPK and PI3K/AKT pathway activation and inhibition in human and canine melanoma. Vet Comp Oncol. doi: 10.1111/vco.12044
77. Freeman KP, Hahn KA, Harris FD, King GK (2003) Treatment of dogs with oral melanoma by hypofractionated radiation therapy and platinum-based chemotherapy (1987-1997). J Vet Intern Med. 17: 96-101.
78. Proulx DR, Ruslander DM, Dodge RK, Hauck ML, Williams LE, Horn B et al. (2003) A retrospective analysis of 140 dogs with oral melanoma treated with external beam radiation. Vet Radiol Ultrasound. 44: 352-9.
79. Murphy S, Hayes AM, Blackwood L, Maglennon G, Pattinson H, Sparkes AH (2005) Oral malignant melanoma - the effect of coarse fractionation radiotherapy alone or with adjuvant carboplatin therapy. Vet Comp Oncol. 3: 222-9. doi: 10.1111/j.1476-5810.2005.00082.x
80. Westberg S, Sadeghi A, Svensson E, Segall T, Dimopoulou M, Korsgren O et al. (2013) Treatment efficacy and immune stimulation by AdCD40L gene therapy of spontaneous canine malignant melanoma. J Immunother. 36: 350-8. doi: 10.1097/CJI.0b013e31829d8a1b
81. Finocchiaro LM, Glikin GC (2012) Cytokine-enhanced vaccine and suicide gene therapy as surgery adjuvant treatments for spontaneous canine melanoma: 9 years of follow-up. Cancer Gene Ther. 19: 852-61. doi: 10.1038/cgt.2012.72
82. Kyte JA, Mu L, Aamdal S, Kvalheim G, Dueland S, Hauser M et al. (2006) Phase I/II trial of melanoma therapy with dendritic cells transfected with autologous tumor-mRNA. Cancer Gene Ther. 13: 905-18. doi: 10.1038/sj.cgt.7700961

83. Alexander AN, Huelsmeyer MK, Mitzey A, Dubielzig RR, Kurzman ID, Macewen EG, Vail DM (2006) Development of an allogeneic whole-cell tumor vaccine expressing xenogeneic gp100 and its implementation in a phase II clinical trial in canine patients with malignant melanoma. Cancer Immunol Immunother. 55: 433-42. doi: 10.1007/s00262-005-0025-6
84. Bergman PJ, McKnight J, Novosad A, Charney S, Farrelly J, Craft D et al. (2003) Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. Clin Cancer Res. 9: 1284-90.
85. Grosenbaugh DA, Leard AT, Bergman PJ, Klein MK, Meleo K, Susaneck S et al. (2011) Safety and efficacy of a xenogeneic DNA vaccine encoding for human tyrosinase as adjunctive treatment for oral malignant melanoma in dogs following surgical excision of the primary tumor. Am J Vet Res. 72: 1631-8. doi: 10.2460/ajvr.72.12.1631
86. Bergman PJ, Camps-Palau MA, McKnight JA, Leibman NF, Craft DM, Leung C et al. (2006) Development of a xenogeneic DNA vaccine program for canine malignant melanoma at the Animal Medical Center. Vaccine. 24: 4582-5. doi: 10.1016/j.vaccine.2005.08.027
87. Ottnod JM, Smedley RC, Walshaw R, Hauptman JG, Kiupel M, Obradovich JE (2013) A retrospective analysis of the efficacy of Oncept vaccine for the adjunct treatment of canine oral malignant melanoma. Vet Comp Oncol. 11: 219-29. doi: 10.1111/vco.12057
88. Mayayo SL, Prestigio S, Maniscalco L, La Rosa G, Arico A, De Maria R et al. (2011) Chondroitin sulfate proteoglycan-4: a biomarker and a potential immunotherapeutic target for canine malignant melanoma. Vet J. 190: e26-30. doi: 10.1016/j.tvjl.2011.02.020
89. Campoli M, Ferrone S, Wang X (2010) Functional and clinical relevance of chondroitin sulfate proteoglycan 4. Adv Cancer Res. 109: 73-121. doi: 10.1016/B978-0-12-380890-5.00003-X
90. Riccardo F, Iussich S, Maniscalco L, Lorda Mayayo S, La Rosa G, Arigoni M et al. (2014) CSPG4-specific immunity and survival prolongation in dogs with oral malignant melanoma immunized with human CSPG4 DNA. Clin Cancer Res. 20: 3753-62. doi: 10.1158/1078-0432.CCR-13-3042
91. Ordas J, Millan Y, Dios R, Reymundo C, de Las Mulas JM (2007) Proto-oncogene HER-2 in normal, dysplastic and tumorous feline mammary glands: an immunohistochemical and chromogenic in situ hybridization study. BMC Cancer. 7: 179. doi: 10.1186/1471-2407-7-179
92. Millanta F, Calandrella M, Citi S, Della Santa D, Poli A (2005) Overexpression of HER-2 in feline invasive mammary carcinomas: an immunohistochemical survey and evaluation of its prognostic potential. Vet Pathol. 42: 30-4. doi: 10.1354/vp.42-1-30
93. Soares M, Correia J, Rodrigues P, Simoes M, de Matos A, Ferreira F (2013) Feline HER2 protein expression levels and gene status in feline mammary carcinoma: optimization of immunohistochemistry (IHC) and in situ hybridization (ISH) techniques. Microsc Microanal. 19: 876-82. doi: 10.1017/S1431927613001529
94. Peruzzi D, Mesiti G, Ciliberto G, La Monica N, Aurisicchio L (2010) Telomerase and HER-2/neu as targets of genetic cancer vaccines in dogs. Vaccine. 28: 1201-8. doi: 10.1016/j.vaccine.2009.11.031
95. Rankin KS, Starkey M, Lunec J, Gerrand CH, Murphy S, Biswas S (2012) Of dogs and men: comparative biology as a tool for the discovery of novel biomarkers and drug development targets in osteosarcoma. Pediatr Blood Cancer. 58: 327-33. doi: 10.1002/pbc.23341
96. Boerman I, Selvarajah GT, Nielen M, Kirpensteijn J (2012) Prognostic factors in canine appendicular osteosarcoma - a meta-analysis. BMC Vet Res. 8: 56. doi: 1746-6148-8-56 [pii] 10.1186/1746-6148-8-56
97. Schmidt AF, Nielen M, Klungel OH, Hoes AW, de Boer A, Groenwold RH, Kirpensteijn J (2013) Prognostic factors of early metastasis and mortality in dogs with appendicular

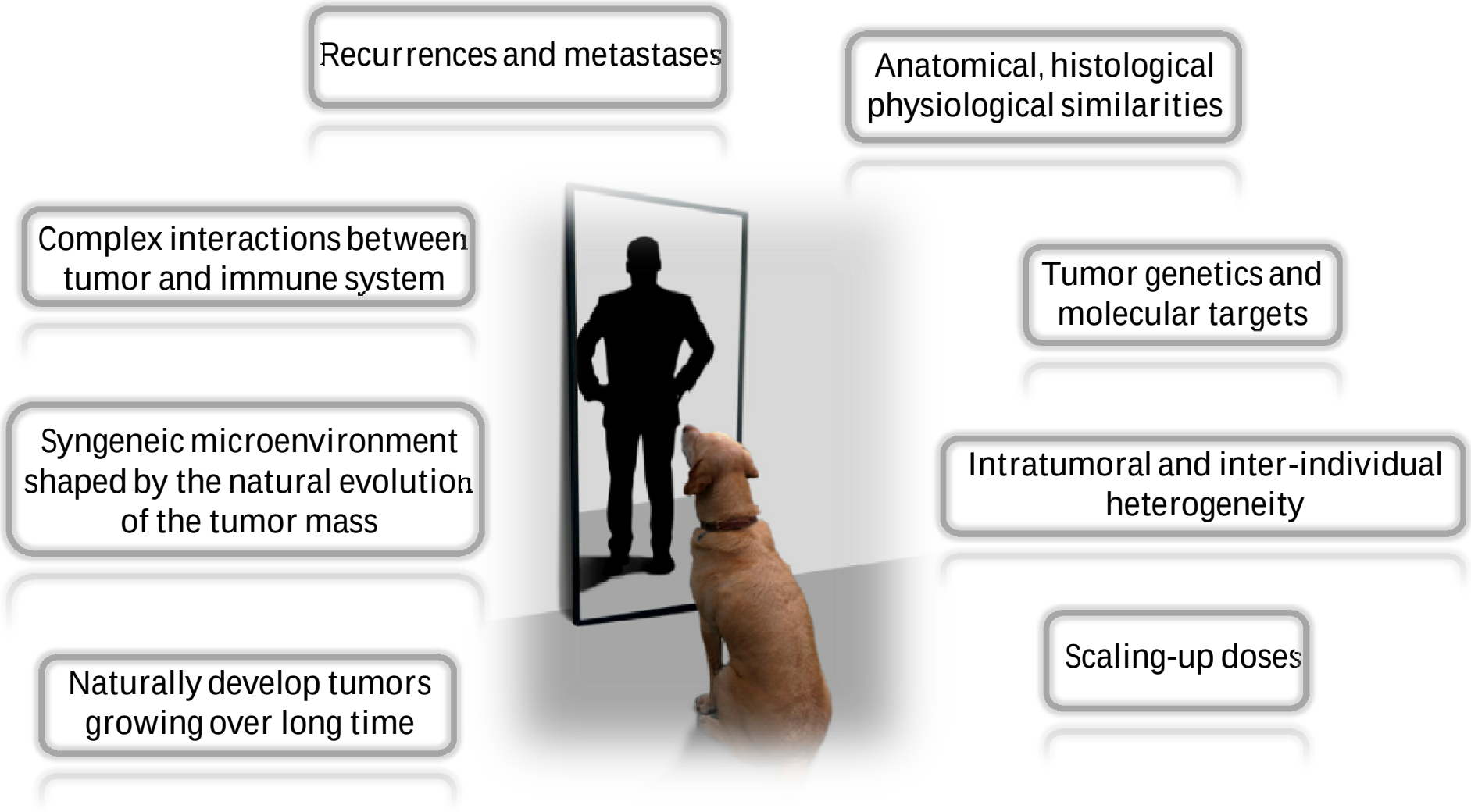
osteosarcoma after receiving surgery: an individual patient data meta-analysis. Prev Vet Med. 112: 414-22. doi: S0167-5877(13)00276-6 [pii] 10.1016/j.prevetmed.2013.08.011
98. Finocchiaro LM, Villaverde MS, Gil-Cardeza ML, Riveros MD, Glikin GC (2011) Cytokine-enhanced vaccine and interferon-beta plus suicide gene as combined therapy for spontaneous canine sarcomas. Res Vet Sci. 91: 230-4. doi: 10.1016/j.rvsc.2010.12.012

Figure 1. Evolution of experimental systems towards 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 towards more comprehensive experimental models (**b**). The advent of genetically engineered mice (GEM) that spontaneously develop tumors and thus recapitulate complete disease evolution provides the first revolution in preclinical cancer research (**c**). To overcome limitations in murine models, testing immunological therapies in large animals, such as nonhuman 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 towards the study of naturally occurring tumors in companion animals which may be priceless comparative models with which to accelerate the entry of new anti-cancer therapies into the human sphere (**e**).

Figure 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 physiological 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 recall the complexity of human cancers thanks to their intratumoral genetic instability and patient heterogeneity. Canine cancer models are of great translational value as avatars of human tumor behavior and therapy response.



Riccardo et al., Figure 1



Riccardo et al., Figure 2