Veterinaria Italiana, **45** (1), 111-120

How companion animals contribute to the fight against cancer in humans

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Summary

Companion animals and their human guardians suffer from many of the same types of cancer and are often treated with many of the same drugs. Moreover, the overall tumour biology is much more similar between humans and companion animals than between humans and rodent tumor models. Therefore, it is proposed that pre-clinical evaluation of novel cancer therapeutics should more often include appropriately designed trials in companion animals with cancer to more accurately predict efficacy and toxicity in humans. For example, studies in dogs with cancer have been used to assess efficacy and design human clinical trials of immunotherapy, gene therapy, sustained release drug delivery and liposomal drug delivery. In the future, such studies will ultimately benefit not only humans, but also companion animals with cancer.

Keywords

Animal, Biology, Canine, Cancer, Chemotherapy, Immunotherapy, Model, One Health, Pet, Public health, Trial, Toxicity, Tumour.

Il contributo degli animali da compagnia alla lotta contro il cancro nell'uomo

Riassunto

Gli animali da compagnia e i loro proprietari presentano molti tipi di cancro in comune e sono spesso trattati con gli stessi farmaci. Inoltre, la biologia tumorale nel suo complesso è molto più simile nell'uomo e negli animali da compagnia che nell'uomo e nei modelli di tumore nei roditori. Si suggerisce pertanto di includere più di frequente, nella valutazione preclinica di nuove terapie anticancro, studi appositamente strutturati sugli animali da compagnia affetti da cancro al fine di prevedere con maggiore precisione la loro efficacia e tossicità nell'uomo. Ad esempio, studi su cani affetti da cancro hanno consentito di valutare l'efficacia, in apposite sperimentazioni cliniche sull'uomo, dell'immunoterapia, della terapia genica, della somministrazione di farmaci a rilascio prolungato e di farmaci liposomiali. In futuro, le conoscenze derivate da tali studi non andranno a vantaggio soltanto delle persone ma anche degli animali da compagnia colpiti da cancro.

Parole chiave

Animale, Animale da compagnia, Biologia, Canino, Cancro, Chemioterapia, Immunoterapia, Modello, Salute pubblica, Studio, Tossicità, Tumore, Una sola salute.

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Introduction

Animals have been used widely to develop and test cancer therapeutics. The vast majority of these studies have been performed using rodent models with transplanted human tumour cell lines. However, lately the value of rodent tumour models for predicting drug treatment outcomes in humans with cancer has been questioned. Indeed, it is not at all clear that rodent tumour models represent the most effective approach to development of new cancer therapeutics. For example, rodent tumour studies typically involve the use of highly inbred animals injected with *in vitro* selected tumour cell lines, after which the animals are maintained under controlled, artificial laboratory environments. In contrast, most tumours of adult humans develop slowly, allowing them to accumulate numerous mutations, many of which are not reflected in cultured tumour cell lines. Moreover, the use of human tumour cell lines grown in immunosuppressed mice ignores the contribution of the immune system and the host stromal cell compartment to overall tumour biology.

Therefore, there has been renewed attention directed towards the use of better animal models that may more accurately predict tumour behaviour and responses to drug therapy in humans. We and others believe that the study of spontaneous tumours in companion animals offers a solution to at least some of these problems (28, 36, 37, 43). Companion animals with naturally occurring tumours provide an excellent opportunity to investigate many aspects of malignancy from aetiology to treatment. Moreover, conducting clinical investigations in companion animals with cancer also provides the opportunity to benefit not only humans, but also the companion animals themselves, since many treatments approved for use in humans are ultimately adopted by veterinary oncologists for use in dogs and cats.

Advantages of the companion animal cancer model

Several aspects of spontaneous cancer in companion animals render this an attractive comparative model for human cancer. For one, companion animals share a common environment with people. Exposure to environmental carcinogens should, therefore, be similar to that in humans (16, 19). In addition, malignancies in companion animals develop spontaneously, whereas experimental laboratory models utilise induced tumours either through exposure to known carcinogens or transplantation of cell culture-derived tumours, often in the presence of artificially induced immune suppression.

Tumours in companion animals generally progress at a more rapid rate than in their human counterparts. However, the time-course is still long enough to allow comparison of response durations, but short enough to ensure rapid accrual of data. Cancers that develop in companion animals also more closely resemble human cancers biologically, including similar cancer cell kinetics and analogous features, such as the development of tumour hypoxia and tumour clonal variation (27, 46). Given the larger body size of companion animals compared to rodents, sample collection (i.e. serum, urine, cerebrospinal fluid, multiple tissue samples), surgical interventions, imaging and the use of novel drug delivery systems can be implemented more easily. Examples of these advantages are illustrated by recent work with inhalational drug and cytokine delivery, which relied extensively on the use of dogs with spontaneous primary and metastatic tumours (20, 25).

Since a single 'standard of care' is typically not well established by veterinarians caring for dogs and cats with cancer, there is also greater protocol latitude allowed in designing prospective clinical trials. Furthermore, it is easier and more ethically acceptable to attempt new and innovative treatment strategies in companion animals with cancer for which there are no good alternatives. It is also far less expensive to conduct clinical trials in

veterinary cancer patients than to conduct similar studies in human cancer patients. Importantly, most companion animal owners are highly committed and actively seeking innovative new therapies for their pet's cancer. For example, compliance with treatment and recheck visits is exceptional, with necropsy compliance approaching 85%, which is significantly better than in most clinical trials in humans.

Cancer incidence in dogs

Over half of all households in the United States include a companion animal, which adds up to approximately 55 million dogs and 60 million cats at risk of developing cancer (43). Cancer is the number one cause of death overall in dogs. Estimates of age-adjusted overall cancer incidence rates in dogs range from 243 to 381 per 100 000 dog/years at risk (8). These rates are comparable to those reported by the National Cancer Institute Surveillance Epidemiology and End Results (SEER) programme for humans, which reports rates in the range of 300 per 100 000 at-risk patients. Rates for some tumour types, such as osteosarcoma, soft tissue sarcomas and lymphoma are significantly higher in dogs than for the same tumour of humans. Thus, their relative abundance increases the value of the model for evaluation of particular human cancers. In the following sections, we will discuss two tumours in dogs that have been used extensively for the evaluation of novel cancer therapeutics for eventual application to humans.

Canine osteosarcoma as a model for bone cancer in humans

Osteosarcoma (bone cancer; OSA) in dogs closely resembles OSA in humans (17, 43). A comparison of the similarities and differences between human OSA and canine OSA is presented in Table I. Canine OSA is a spontaneous tumour that primarily affects large to giant breeds of dogs. The majority of dogs are diagnosed when the tumour affects their long bones, especially the humerus, radius, or femur. Most OSA tumours in dogs are high-grade tumours and present with extra-compartmental (stage IIB) disease. The only known (negative) prognostic factor identified for dogs with OSA to date is the presence of elevated serum alkaline phosphatase at the time of diagnosis and failure of elevated concentrations of alkaline phosphatase to decrease following amputation (14, 15).

Dogs with bone cancer that are treated with amputation alone typically survive a median of 3-4 months, with death commonly occurring due to lung metastasis. Only 10% of dogs treated with surgery alone survive for more than one year (7). Adjuvant chemotherapy using platinum-based drugs, such as cis-platinum results in median survival times (MST) of 10-12 months (3, 40). However, 80%

Table I
Comparison of canine and human osteosarcoma

Variable	Dog	Human
Incidence in the United States	8 000-10 000/year	2 000/year
Mean age	7 years	14 years
Gender	1.5:1 male:female	1.5:1 male:female
Body size	Large to giant breeds	Heavy
Site	80% appendicular	90% appendicular
Percent without metastasis at presentation	80-90%	80-90%
Percent high grade	95%	85-90%
Metastatic rate without chemotherapy	90%	80%
Sites of metastasis	Lung, bone, soft tissue	Lung, bone, soft tissue
Improved outcome with chemotherapy	Yes	Yes

of treated dogs still die of metastasis by 24 months following chemotherapy.

Immunotherapy trials in dogs with osteosarcoma and direct translation to treatment of humans

Over 150 dogs with OSA have been evaluated in studies using the immunotherapy drug liposome muramyl tripeptide phosphatidyl-ethanolamine (L-MTP-PE), a potent stimulator of innate immunity (26, 30, 31). Significantly improved outcomes were found in a randomised, placebo controlled post-surgical trial in which dogs with appendicular OSA were randomised to receive L-MPT-PE or placebo liposomes. Dogs receiving L-MTP-PE had a MST of 7 months, as opposed to 3 months for those dogs receiving placebo liposomes (30). However, 70% of these dogs still died of metastases. Therefore, a follow-up study was performed, in which dogs with OSA were initially treated with amputation plus cisplatin chemotherapy, which was followed by treatment with L-MTP-PE or with placebo liposomes (26). Dogs that received chemotherapy and then L-MTP-PE had a significantly longer MST (14.4 months) than dogs receiving placebo liposomes (MST: 9.7 months).

Based largely on the results of the studies performed in dogs with bone cancer using L-MTP-PE, clinical studies were initiated in humans with OSA. One of the largest studies was a randomised, placebo-controlled clinical trial of surgery and chemotherapy, followed by L-MP-PE immunotherapy, which was based on the design of the original clinical trial in dogs with OSA (33, 34). In this trial, a chemotherapy regimen-dependent increase in disease-free and overall survival was observed, as predicted by the proof-of-concept canine studies.

Inhalational delivery of cytokines for immunotherapy of cancer metastases

Dogs with OSA and other tumour lung metastases were used in another study to evaluate the safety, efficacy and immunological effects of inhaled interleukin-2 (IL-2) liposomes (24, 25). In one study, significant anti-tumour responses were observed in 2 of 4 dogs with OSA, accompanied by significant increases in total leukocytes and effector cell cytolytic activity in bronchoalveolar lavage samples following treatment (25). These studies in turn helped lead the way to the use of inhalational delivery of liposomal IL-2 in human patients with metastatic cancer (39).

Evaluation of cisplatin drug delivery systems in dogs with osteosarcoma

Studies have also been conducted in dogs to evaluate new drug delivery systems that can release a very high dose of chemotherapy into a surgical wound while also eliciting slow release of relatively low concentrations of chemotherapy systemically (41). One such system is a biodegradable polymer called 'open cell polylactic acid containing cisplatin' (OPLA-PT). When OPLA-PT was implanted in normal dogs, no systemic toxicity and no retardation of bone allograft healing were identified at doses of up to 80.6 mg/m^2. Serum pharmacokinetic data following OPLA-PT implantation revealed a roughly 30-fold increase in the area under the curve (AUC) for systemic platinum concentration compared to similar doses administered intravenously. Therefore, a clinical trial was conducted in 39 dogs with OSA that were treated with amputation plus implantation of OPLA-PT at the surgical amputation site (41). The MST for dogs treated in this study was 8 months, with a one-year survival rate of 41.2%. These results compare favourably to treatment with multiple doses of cisplatin administered intravenously, but with much less treatment-associated toxicity.

Another study evaluated the efficacy of liposome-encapsulated cisplatin as a post-surgical treatment for canine OSA (44). The efficacy of adjuvant STEALH® liposome-encapsulated cisplatin compared to 'standard-of-care' carboplatin therapy was evaluated in dogs in a randomised clinical trial. While liposome encapsulation of cisplatin allowed

the safe administration of five times the maximally tolerated dose of free cisplatin to dogs without requiring the concurrent use of fluid support or anti-emetic drugs, this treatment approach did not lead to any significant increase in disease-free survival. However, a larger proportion of dogs receiving the liposomal cisplatin experienced long-term disease free survival when compared with dogs receiving standard carboplatin chemotherapy (44).

Evaluation of intravenous gene therapy in dogs with osteosarcoma

The first studies evaluating the use of intravenously administered gene therapy in large animals were conducted in dogs with metastatic OSA (13). In those studies, a safe and effective dose of liposome-plasmid DNA complexes encoding the IL-2 gene was determined. In 22 dogs enrolled in the study and treated by intravenous administration of lipsome-DNA complexes, significant activation of innate immunity was observed, together with a significant increase in survival times compared to historical control animals. In a subsequent study of intravenous gene delivery in dogs with soft tissue sarcoma, it was found, based largely on prior mouse studies, that most of the anti-tumour activity elicited by the treatment was in fact due to activation of innate immunity by the liposome-DNA complexes themselves (11, 12, 23). Based in part on the results of these studies in dogs, the liposome-DNA complex technology has been developed as an immunotherapeutic. Clinical trials of liposome-DNA complexes as vaccine adjuvants and as antiviral and anticancer immunotherapeutics are currently underway in humans (9).

Canine malignant melanoma as a model for humans with melanoma

The oral cavity of dogs is a common site for the development of a variety of malignant and benign tumours. Malignant melanoma is the most common oral malignancy in dogs. Oral melanomas in dogs are highly malignant tumours, with metastasis occurring rapidly via lymphatics or blood vessels to regional lymph nodes, lungs, liver, brain and kidney. Following complete surgical removal of the primary tumour, approximately 25% of dogs with oral melanoma will survive one year or more (18). The major recognised prognostic factors for dogs with melanoma are tumour size, presence of lymph node metastasis, and the ability of the first surgery to afford local control (18, 29).

Treatment of oral melanoma in dogs with single-agent melphalan or carboplatin yields objective response rates of approximately 20-25% (35, 38). Local coarsely fractionated radiotherapy results in a high local response rate, but the rapid development of metastatic disease remains problematic (2, 6). However, as is the case with melanoma in humans, there remains a major need to develop new approaches to prevent or delay the development of tumour metastases.

Evaluation of non-specific tumour immunotherapy in dogs with melanoma

Melanomas are known to be more immunogenic than most other tumours. Therefore, immunotherapy has been widely viewed as an attractive treatment option for melanoma in both humans and dogs. In one of the earliest immunotherapy studies conducted in dogs, dogs with melanoma were treated with adjuvant immunotherapy using heat-killed *Corynebacterium parvum* (29). In that study, it was observed that in dogs with advanced tumours, treatment with immunotherapy plus surgery resulted in a significant improvement in survival compared to surgery alone. In a second study of immunotherapy in canine melanoma, 98 dogs stratified by stage were randomised to receive placebo liposomes, L-MTP-PE, or L-MTP-PE plus recombinant canine granulocyte-macrophage colony-stimulating factor (GM-CSF) (22). Dogs with stage I disease receiving L-MTP-PE had a significantly improved outcome versus dogs receiving placebo (32).

In a more recent study, the ability of local tumour transfection with potent immune stimulatory genes (the *Staphylococcus* enterotoxin B gene plus the IL-2 gene) to stimulate anti-tumour immunity was evaluated (10). In that study, an overall response rate of 46% was reported, with significant prolongation of survival in patients with stage III tumours compared with historical controls treated with surgery alone. In some treated patients, dramatic tumour regression was noted over a period of several weeks following intra-tumour gene delivery (Fig. 1). Clinical responses in dogs treated with superantigen gene therapy were also associated with a significant induction of tumour-specific cytotoxic T lymphocyte activity, along with lymphocytic infiltrates in the tumour. The use of superantigen gene therapy was also evaluated in dogs with soft tissue sarcoma (42). These results led directly to the initiation of a subsequent phase I clinical trial of superantigen-cytokine gene therapy in human patients with malignant melanoma (45).

Melanoma vaccine studies in dogs

A number of tumour-specific antigens have been identified in human melanomas and many have the potential to serve as antigens for the development of tumour vaccines. Tumour vaccines have also been evaluated in dogs with melanoma and other tumours. In one approach, the efficacy of human GM-CSF transfected autologous tumour cell vaccines for the treatment of advanced melanoma was investigated in dogs with melanoma (21, 22). Vaccination was well-tolerated and objective tumour responses were noted in 19% of vaccinated dogs. A positive response was often associated with a T cell infiltrate in the tumour, and delayed-type hypersensitivity conversion in skin injection sites was observed in several cases. A subsequent clinical trial evaluated the efficacy of an allogeneic, whole-cell canine melanoma vaccine that was engineered to over-express the human melanoma antigen gp100 antigen (1). Objective tumour responses were noted in 17% of vaccinated dogs in that study.

More recently, a plasmid DNA vaccine encoding a xenogeneic melanoma antigen (human tyrosinase) has been evaluated in dogs with melanoma (4, 5). Side-effects from the

Figure 1
Tumour response to intratumoral superantigen-cytokine gene therapy in a dog with oral malignant melanoma
A dog with oral melanoma was enrolled in a clinical trial of superantigen (SEB) and cytokine (IL-2) gene therapy (top panel, pre-treatment)
Treatments were administered by direct intra-tumoral injections of liposome-DNA complexes encoding SEB and canine IL-2 on an every other week basis
Beginning within two weeks of the first injection, significant depigmentation of the tumour was noted (middle panel)
Subsequently, the tumour began to decrease in size and completely regressed after two injections (week 4, bottom panel)
The tumour did not recur during the next two years of follow-up evaluation

vaccine have been minimal to date and a few objective tumour responses were noted. Comparison with historical control data suggested a survival benefit, although more complete assessment of vaccine efficacy awaits the results of ongoing clinical trials. Nonetheless, the vaccine has been approved for use in dogs with melanoma. Thus, the canine tyrosinase-based melanoma vaccine represents the first licensed vaccine for the treatment of cancer in dogs or humans.

Conclusions

The discussion supports our contention and that of others that dogs with spontaneous cancer represent an important and underutilised animal model for the evaluation of cancer therapeutics (28, 36, 37, 43). We can expect that translational studies of cancer therapeutics in dogs will become more common as the value of dogs as a cancer model becomes more widely accepted. It is important to also realise that in addition to human cancer patients, dogs and the pet-owning public often benefit directly from these studies, since many of the drugs developed in this manner often find their way back to canine patients. Thus, translational cancer studies in dogs truly represent the best of the principles envisioned by the 'One Health, One Medicine' concept.

Further *in vitro*, *ex vivo* and *in vivo* comparative studies of the biology and therapeutic response of canine and human tumours, through gene expression, molecular, cell-based and clinical studies, will strengthen the value of the canine spontaneous tumour model. This is one of the major goals of the Comparative Oncology Program, housed within the Center for Cancer Research at the United States National Cancer Institute (ccr.cancer.gov/resources/cop/). In addition to comparative biology studies of canine and human cancer, the Comparative Oncology Program seeks to validate cross-reactive diagnostic reagents for use in companion animal cancer research, and co-ordinates a group of academic veterinary oncology programmes (the Comparative Oncology Trials Consortium) that participate in pharmacodynamically intensive, proof-of-concept clinical trials of novel cancer therapeutic agents with human oncology application. It is hoped that the studies being conducted through the Comparative Oncology Program and at other academic veterinary institutions will lend further credence to the utility of animals with cancer as models for the human condition.

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