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Spontaneous and genetically engineered animal models: use in preclinical cancer drug development

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Abstract

The preclinical development of anticancer drugs has been based primarily on the transplantation of murine or human cancers into mice. Alternatives to these transplantation models are animals that naturally develop cancers with features relevant to the human disease. The first group of these models arises in mice that are genetically engineered to develop cancer. The second group includes pet dogs and cats that naturally develop cancer. This review will discuss the use and integration of these spontaneous cancer models into a comprehensive and comparative approach to preclinical drug development. Examples of their successful use and an outline of their relative strengths and weaknesses will be provided.

Keywords: Cancer; GEM; Mouse; Transgenic; Canine; Comparative; Spontaneous

1. Introduction

Advances in molecular biology and genetics have propelled our understanding of the biology of cancer. Unfortunately, these discoveries have well out-paced the application of this information to the human condition of cancer. Limiting translation from the preclinical setting to the clinic has been the availability of relevant *in vivo* cancer models. As novel biology-based therapeutics enter the drug development pipeline the need for more sophisticated and informative preclinical models has and will continue to increase. Lacking from most preclinical approaches is the use of models that are characterised by spontaneous cancer development in immune competent and syngeneic hosts. Examples of such models include genetically engineered mice (GEM) and companion (pet) animals that naturally develop cancers. It is important to recognise both the strengths and the weaknesses of these models such that they may be appropriately used and integrated into a comprehensive drug-development programme. Spontaneous cancer models offer an opportunity to apply a 'comparative' perspective to the discovery and development of new drugs that is based on a diverse group of well-defined and well-characterised animal models.

2. Genetically engineered mice (GEM)

When first introduced, GEM that developed cancers were characterised by rapid cancer progression, the development of primarily haematopoietic malignancies and/or synchronous tumour development at multiple anatomical sites [1,2]. These models provided limited opportunities for preclinical assessment of novel therapeutics. Recent advances in modelling strategies have resulted in mice that develop cancers in a diversity of organs with an ordered progression that more closely resembles the human cancer condition. Strategies used to develop cancer-prone GEM include conditional expression of genes of interest in specific tissues or during specific phases of development, and/or the use of selective somatic expression to allow the expression of genes within limited numbers of 'target' cells. A detailed discussion of these advances in modelling is outside the scope of this review and has been summarised recently [1,3]. Models are continually being refined, and for many cancers there now exist mice which develop

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spontaneous tumours that have histological similarities to their human counterparts, share molecular and genetic features, and are associated with relevant tumour-microenvironment interactions in an intact immune system. In some cases these models are associated with spontaneous metastases to distant sites. Information on the construction, availability and phenotype of these mice is now available through the National Cancer Institute-sponsored Mouse Models of Human Cancer Consortium (www.emice.nih.gov). The unique features of GEM provide opportunities for the evaluation of novel therapeutics not possible through conventional transplantation and xenograft murine models.

Historically the use of GEM mice in translational research has focused on the immunobiology of cancer [4,5]. In practice there have been very few examples of the use of GEM in the preclinical development of novel therapeutics. Table 1 provides examples of GEM that have been used or are amenable for use in this purpose. The limited use of GEM in preclinical drug development may in part relate to the spontaneous nature of tumour development seen in these mice. Most GEM undergo carcinogenesis and tumour formation during an 'at risk' period of time that is specific for each GEM. This process is not synchronised between mice, resulting in differences in the age and time when individual mice in a group develop tumours. In some models, such as the RIP-TAG model of pancreatic cancer, defined histological lesions have been described over relatively narrow ages of mice. For example, hyperplastic pancreatic islets are present at 3–5 weeks of age, with progression to angiogenic lesions at 9–10 weeks, and solid tumours at 12 weeks [6]. These narrowly defined lesion times (age groups) allow treatment of mice in a variety of settings: (i) prevention of tumour development, (ii) delay of tumour progression; or (iii) treatment of measurable carcinomas [6]. However, in many GEM, cancers develop over relatively long 'at risk' periods, for example the p53+/−mouse may develop tumours over 7–17 months [7]. This protracted period of risk is problematic in the design of experiments that test novel drugs. A further complication relates to the fact that most p53±mice will develop tumour-associated morbidity soon after the primary tumour is detected, thereby limiting treatment opportunities for the evaluation of new drugs. In cases where the 'at risk' period is prolonged, the use of novel biomarkers for tumour development in the GEM may be helpful and essential. Examples of such biomarkers may include analysis of circulating cells or proteins, tumour genomics or proteomics, and imaging. Techniques for bioluminescence imaging, magnetic resonance imaging and positron-enhanced tomography exist or will soon exist that will allow rapid screening of large groups of GEM to define tumour development or progression [8–11]. These same imaging strategies have the advantage of defining endpoints of response during the assessment of a drug intervention.

Three broad characterisations of experimental approaches that have been or may be considered in the efficient use GEM during preclinical drug development are presented in Fig. 1. The first approach (Fig. 1a) involves the prevention of tumour development or tumour progression following simultaneous treatment of GEM within an experimental group. Comparisons can be made between drug-exposed and age/sex-matched controls. Endpoints may include tumour development, tumour progression, or in some cases objective responses against measurable tumours. This experimental approach is best suited to GEM that have well-defined endpoints or where endpoints are narrowly defined by age or another surrogate marker. As discussed above, the RIP-TAG GEM of pancreatic cancer has been used effectively to evaluate novel anti-angiogenic therapies against endpoints of tumour development, tumour progression, and regression of established pancreatic tumours [6,12]. In this example, as with all other GEM, the fact that tumour, tumour-microenvironment and host are all syngeneic is significant and potentially necessary for the evaluation of novel biotherapeutic agents such as antiangiogenics. Models such as the RIP-TAG may be made even more valuable when coupled to bioluminescent imaging [13].

For GEM with less well-defined disease endpoints the approach outlined in Fig. 1(b) may be more appropriate. This strategy involves the treatment of GEM in an experimental design that has similarities to a clinical trial. Each mouse is examined for tumour or lesion development based on physical examination or a surrogate marker. The therapeutic intervention is then initiated in that mouse and the course of the disease is followed using physical examination or a surrogate endpoint in that mouse. Individualised timing of therapy for each mouse dramatically increases management effort and experimental costs when compared with more conventional models involving the simultaneous treatment of groups of mice. Furthermore, tumours must be detected before their size precludes therapeutic intervention. Omer and colleagues utilised this type of approach by treating mammary tumours in Ki-Ras transgenic mice using farnesyl transferase inhibitors once tumours reached a predetermined volume [14]. The logistical complexity of managing this 'clinical trial'—like experiment may have precluded its widespread use in the evaluation of novel therapeutics in GEM.

In models where variability in the time to tumour development is significant or where disease progression is rapid, the strategy outlined in Fig. 1(c) may be considered. This approach involves the use of GEM as a source for transplantable cancer tissues or cell lines. GEM mice are monitored for tumour development and

Table 1
Examples of companion animal cancers or genetically engineered mice that have been used or are amenable for use in the preclinical development of drugs

Non-Hodgkin's lymphoma

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 15.5-29.9/100 000 [18]	• Diffuse large B-cell most common (high grade) • Follicular and nodular next most common (low grade) • NCI Working Formulation useful in grading	• Nodal or extranodal at presentation • Median survival 5 years [18]	• CHOP-like[b] chemotherapy most common • Involved field and/or total body irradiation (TBI)	
Canine	• 15-30/100 000 [59]	• Diffuse large B-cell most common (high grade) • NCI Working Formulation is useful in grading • T-cell seen in 10–38% [23,25]	• Multicentric nodal presentation most common • Median survival 1 year	• CHOP-like chemotherapy	• Used to develop TBI regimen [37] • Liposomal L-asparginase [60,61] • Intralymphatic autologous lymphoma tumour cell vaccine [62] • *Streptococcus marcescens* and *S. pyogenes* mixed vaccine [63] • Antiangiogenic thrombospondin-I peptide (ABT526)[c]
Murine	• *p16(Ink4a)* null	• Develop thymic hyperplasia • Spontaneously develop lymphoma and sarcoma [64]	• Null allele of *p16(Ink4a)* gene • Normal p19(Arf) function • Augmented T-cell proliferative response • Median time to tumour development 19 months [64]		
	• P190	• BCR/ABL • Resembles human Ph-positive acute lymphocytic leukaemia [65]	• 95% die of leukaemia or leukaemia/lymphoma by 1–7 months [65]		• Farnesyl transferase inhibitor SCH66336 [66]
	• Msh2 knockout	• Lymphoma most common • Also develop intestinal and skin neoplasms	• Deficient in *Msh2* mismatch repair gene • 80% of mice develop lymphoma • Develop tumours 2–24 months [67]		

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Table 1 (*continued*)

Leukaemia					
	Incidence or Prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 9.7/10,00 all types • 2.5/100 000 AML[d] • 2.3/100 000 CLL[e] • 1.3/100 000 ALL[f] • 1–2/100 000 CML[g] [68]	• 25% of leukaemias are AML	• AML: 30–50% achieve CR • CLL: 30–70% respond to therapy • ALL: 65–85% achieve remission; 35–45% achieve long-term survival • CML: 5–7 year median survival [68]	• Specific treatments depend on histological subtype • Bone marrow or stem cell transplant • Myeloablative and non-myeloablative chemotherapy • Antibody (e.g. Irbitux)	
Canine	• Unknown (rare) (58)	• ALL most common	• ALL: poor prognosis, few dogs survive longer than 2 months [25]	• Vincristine, prednisone, cyclophosphamide chemotherapy • Hydroxyurea (CLL) • CLL may be chemotherapy-responsive	• L-asparginase and liposomal L-asparginase [60,61]
Murine	• PML-RARα • PML-RARα with FLT3 mutation	• Acute promyelocytic leukaemia [15]	• Human fusion gene *PML-RARα* cloned into hMRP8 expression cassette • Impaired neutrophil maturation • PML-RARα develop leukaemia at 8.5 months • PML-RARα/FLT3 mutants develop leukaemia at 3–10 months [15]		• Retinoic acid [15] • Receptor tyrosine kinase inhibitor (targets FLT3) [69]
	• Nf1±	• Subset develop myeloid leukaemia • Subset develop lymphoma [70]	• Non-functional *Nf1* tumour suppressor gene • Myeloproliferative disorder (MPD) similar to juvenile myelomonocytic leukaemia (JMML) • Associated with leukocytosis, splenomegaly and hyperactive Ras • About 10% develop disease [70]		• Farnesyl-transferase inhibitors [70]
	• PLZF-RARα	• Chromosomal translocation found in acute promyelocytic leukaemia (APL) • Phenotype resembles human CML more than APL	• PLZF-RARα fusion protein expressed in myeloid-promyelocytic lineage • Die of leukaemia between 6–18 months [72]		• Retinoic acid (RA) [71] • As2O3 [71]
	• PLZF-RARα×RARα-PLZF	• Model of t(11;17) APL • Mimics APL phenotype [73] • BCR/ABL • Resembles human Ph-positive ALL [65]	• Blast/promyelocytic cells accumulate in the bone marrow and spleen without leukocytosis. • 100% develop leukaemia by 6 months of age [73]		• Histone deacetylase inhibitor [73]
	• P190	• Can also develop lymphoma	• 95% die of leukaemia or leukaemia/lymphoma by 1–7 months [65]		• Farnesyl transferase inhibitor (SCH66336) [66]

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Colorectal

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• Colon: 30.3/100 000	• 90–95% are adenocarcinomas • Multistep carcinogenesis with corresponding histological progression [68]	• 61% 5-year survival rate in USA • 90% survival at 5-years for disease confined to mucosa/submucosa [68]	• Early stages curable with surgery • Invasive or advanced disease associated with poor prognosis	
Canine [59]	• Rare	• Carcinoma • Most commonly rectal in origin [59]	• 1–2 year mean survival [59]	• Surgery • Tumour size and invasiveness predicts surgical success	
Murine	• APC (min)	• Multiple adenomas of small intestine [74]	• Mutated APC • Similar to familial adenomatous polyposis and sporadic cancer • Rapid development of polyps • Adenocarcinomas are uncommon • No K-*ras* mutation or p53 inactivations [74]		• Cox-2 inhibitor [75] • Antiproliferative herbs (PC-SPES) [76]
	• APC1309	• Intestinal adenomas [77]	• Similar to familial adenomatous polyposis • Nonsense mutation of APC at codon 1309 • Average death at 4 months [77]		• Cox inhibitors [78] • Prostaglandin E receptor antagonists [79] • α-glycosidase inhibitor [77]
	• APC1638n/CEA	• Carcinoma [80]	• Cross of APC1638N knockout mouse crossed with gastrointestinal (GI) CEA transgenic • Develop GI polyps in 6 months • Develop invasive disease around 8 months [80]		

Prostate cancer

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical Applications
Human	• 136/100 000 [68]	• Glandular/acinar origin • Gleason Score/Sum: scoring for multiple foci and glandular pattern • High-grade prostatic intraepithelial neoplasia (HGPIN) present in 85% of men with prostate cancer [54]	• Local disease at diagnosis • PIN as a precursor [23,54] • 95% 5-year survival for stages • Progression-associated hormonal independence • Metastasis to regional lymph nodes, bone, lung, other [68]	• Radical prostatectomy • Adjuvant radiotherapy • Chemoresistant following progression to hormone independence	
Canine	• Unknown • Necropsy study: 1/150 dogs over age of 8 years have advanced prostate cancer [23]	• High-grade carcinoma • Gleason Score/Sum: not applied or validated • HGPIN present in 55% of elderly sexually intact dogs without prostate cancer [54]	• Advanced/invasive at diagnosis • Median survival 30 days without treatment • PIN may be a precursor [23,54]	• Prostatectomy not successful • Chemoresistant • Radioresistant	• Chemoprevention (selenium) [56]

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Table 1 (*continued*)

Prostate cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical Applications
		• HGPIN present in 66% of pet dogs with prostate cancer [54] • Potentially ductal in origin [55]	• Hormone independent at diagnosis • Metastasis to regional lymph nodes, bone, lung, other • Dog is only spontaneous animal model of prostate cancer		
Murine	• TRAMP	• Adenocarcinoma of prostate [81]	• Prostate tumours in 100% • Average tumour onset at about 6 months • Average age at death about 8 months [81]		• Antiangiogenic small-molecule inhibitor (SU5416) [82]
	• G gamma T-15	• Start as high-grade PIN • Progress to advanced metastatic carcinoma [83]	• Human fetal globin promoter linked to SV40 T antigen (Tag) • Develops androgen-independent prostate cancer [83] • Develop prostate tumours 16 weeks • 75% progress to prostate cancer		• Vitamin D analogue (EB 1089) [83]
	• C3(1)/SV40 Tag	• Male mice develop low-grade PIN at 3 months [84]	• Progress to HGPIN at 5 months • Invasive carcinomas appear after 7 months • About 40% of mice surviving to 9 months develop invasive carcinoma • Metastases rare [84]		• 2-difluoromethylornithine and dehydroepiandrosterone [84]
Breast cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 108.8/100 000 [18] • 30% of female cancers [23]	• Ductal carcinoma *in situ* (CIS) • Lobular carcinoma CIS [18]	• Risk is related to lifetime oestrogen exposure • Localised and regional at diagnosis • 60% of are oestrogen receptor positive • c-*erb*B-2 overexpression [18] • 86% 5-year survival [85]	• Modified radical or conservative mastectomy • Radiotherapy or chemotherapy added with advanced stage • Herceptin	
Canine	• 198.9/100 000 [18] • Reduced prevalence following ovariohysterectomy (OHE)	• 50% mammary tumours are benign • Malignant: carcinoma most common; probably lobular in origin [18]	• Early reduction in oestrogen exposure (OHE) is protective • Canine sex-hormone cycle is distinct from humans [23] • 45% Oestrogen-receptor positive	• Surgical resection Doxorubicin for non-resectable tumours	• BCG combined with surgery [87] • Immunotherapy (L-MTP-PE)[h] [88] • Leutinising hormone releasing hormone analogue [89]

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Table 1 (*continued*)

Breast cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
			• Overexpression of ErbB-2 in 17/23 malignant mammary tumours [86] • No BRCA1/2 families [18] • Median survival 22 months with surgery alone [59]		
Murine	• Wap-ras/F	• Carcinoma [90]	• DMBA induces tumours in 100% of mice by 7 months [90]		• Farnesyl protein transferase inhibitor (SCH66336) • Recombinant adenovirus expressing p53 tumour suppressor (SCH58500) • Difluoromethylornithine (DFMO) [91]
	• C3(1)SV40T/t-antigen (Tag)	• Progression to invasive carcinomas [92]	• Over-expressed early region of SV40 induces tumours via Tag inactivation of p53 and Rb • Mammary intraepithelial neoplasia at 3 months • Invasive carcinomas after 4 months • Subset develop metastases to lung • Oestrogen-receptor negative and oestrogen independent [92]		• Cox inhibitors • 9-*cis* retinoic acid • Ornithine decarboxylase inhibitor • Dehydroepiandrosterone [92]
	• MMTV-Ki-Ras [14]	• Mammary adenocarcinomas [14]	• Ki-ras with activating mutation under control of mouse mammary tumour virus promoter/enhancer • Develop tumours starting at 2 months • Average tumour latency 3–8 months • Metastasise occasionally [14]		• Farnesyl protein transferase inhibitors [14] • Geranylgeranyl protein transferase type I inhibitors (GGPTase-I) [93]
	• MMTV/neu + MMTV/TGFα [94]	• Multifocal tumours • Epithelial dysplasia progresses to mammary adenocarcinoma [94]	• Mammary tumour virus-driven by *neu* transgene • 95% develop tumours by about 8 months [94]		• Epidermal growth factor receptor inhibitor (AG-1478) [95]
	• MMTV/PyMT	• Multifocal tumours • Carcinomas [96]	• Middle T oncogene under control of mouse mammary tumour virus promoter/enhancer • Metastasis to lung [96]		• TGF-β inhibitor: Fc:T(β)RII [97]

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Table 1 (*continued*)

Lung carcinoma

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 54.2/100 000 [68]	• Non-small cell lung cancer most common (carcinoma, adenocarcinoma, large cell carcinoma) [23] • Small cell lung cancer (oat cell, intermediate cell, combined oat cell) account for 18% of diagnoses [68]	• Common metastasis sites: pleura, lung, bone, brain, pericardium, liver [68] • 15% 5-year survival overall [85]	• Surgery, radiation • Chemotherapy	
Canine	• 4.17/100 x000 (58)	• Adenocarcinoma most common [59]	• Advanced disease at diagnosis • Mutations in K-*ras* identified [98] • Survival <2 months following surgery if lesion >5 cm or metastatic • Survival >1 year if lesion <5cm and not metastatic [25]	Surgery	• Interleukin-2 inhalation [36] • Inhalational chemotherapy [99]
Murine	• P53/INK4a/ARF	• 80% Adenocarcinoma	• Dominant negative p53 mutation • Ink4a/ARF heterozygous-deficient • Lung tumours carcinogen-induced [100,101]		• Budesonide: synthetic glucocorticoid for chemoprevention [100] • Farnesyl transferase inhibitor [101]
	• UT7.1 [102]	• Lung adenocarcinomas • Stomach tumours • Salivary gland tumours • Pancreatic tumours [102]	• Hybrid gene with 4.7 kb of the rabbit uteroglobin 5′-flanking sequences fused to SV40 T antigen-encoding region • Variable time course for tumour development [102]		

Head-and-neck carcinoma

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical Applications
Human	• Oral cavity, pharynx 10.0/100 000 • Larynx about 7/100 000	• Squamous cell, adenocarcinoma, verrucous carcinoma [103]	• Larynx, oral cavity, thyroid most common • 64% 5-year survival overall [103]	• Surgery$\pm$irradiation	
Canine	6% of canine cancers [25]	• Squamous cell carcinoma • Adenocarcinoma	• Primarily oral and nasal • Locally invasive, slow to spread to distant sites • Survival dependent on histology [25]	• Surgery$\pm$irradiation	• Gene therapy [104] • Sustained release local platinum delivery (OPLA-Pt) [105]

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Table 1 (*continued*)

Head-and-neck carcinoma					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical Applications
Murine	UT7.1 [102] L2D1(+)/p53(±) [106]	• See above • Invasive oral-oesophageal cancer [106]	See above • Severe oral-oesophageal squamous epithelial dysplasia at 5 months [106]		• Sulindac [106]
	P53±[107]	• Oesophageal squamous cell carcinoma in 100% treated with DBN; TCC in >50% of mice treated with DBN [107]			
Liver cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	4.2/100 000 [68]	• Often associated with cirrhosis • Associated with hepatitis B/C [68]	• 5% survival at 5 years • Growth pattern associated with outcome: hanging type, pushing type, infiltrative type [68]	• Potentially curative: partial or total hepatectomy • Palliative: resection, chemotherapy, immunotherapy, hormone therapy	
Canine	Rare [25]	• No association noted with viral exposure • Hepatocellular adenoma and carcinoma most common [25]	• Locally invasive, slow to metastasise to distant sites [25]	• Surgical resection	
Murine	• (C57BL/6 X SJL/J)F1	• Adenoma • Carcinoma [108]	• Carry the metallothionein-ovine growth hormone fusion gene • Incidence about 70% after 11 months [108]		
	• X/myc	• Hepadnavirus-related hepatocarcinogenesis	• Early premalignant dysplastic hepatocytes • 50% have liver tumours by about 10 months • Preneoplastic increase in expression of c-*myc* • Increased hepatocyte proliferation [109]		• High-dose interferon (IFN)-α therapy [109]
	• T-SV40 [109]	• 100% penetrance • Hepatocellular carcinoma	• Cytolysis as early as 1 month • Progression from hyperplasia to proliferative nodules of differentiated cells • Occasional lung metastases [110]		• Adenovirus IFN-γ [111]

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Brain

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• CNS tumours: 2-19/100 000 • Spinal: intracranial 15:1000 [68]	• Astrocytomas, glioblastoma, meningioma most common [68]	• Low grade astrocytoma: 21–55% survival at 5 years • Glioblastoma multiform • 6% 3-year survival [68]	• Surgery • Radiation for infiltrative tumours • Chemotherapy	
Canine	• 14.5/100 000 [25]	• Glioma and meningiomas most common • Usually solitary [25]	• Locally invasive [25]	• Surgery • Radiation	• Brachytherapy [112] • Hyperthermia [113] • Gene therapy [114]
Murine	• Ptc+ [115] • Ptc±p53−/−[116] • GFAP/v-src [117]	• Medulloblastoma [116]	• 15% of Ptc +/−develop by 10 months • 98% of Ptc+/−p53−/−develop by 3 months [116]		
		• Form astrocytomas • Low grade or anaplastic • Can be similar to glioblastomas [117]	• Express v-*src* from GFAP promoter • Most develop dysplastic changes early • 15% develop malignant astrocytoma after 16 months [117]		
	• GFAP/H-ras (emice)[i]	• Astrocyomas with characteristics of glioblastomas	• Overexpress H-*ras* from GFAP promoter		
	• Nf-1/p53−/−(emice)[i]	• Astrocytic tumour similar to human glioblastomas	• Combined mutation: p53 and Nf-1		
	• Mutated EGFR, INK4a-/- [118]	• Glioma	• Similar to human glioma [118]		

Bladder cancer

	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 16.2/100 000 [68]	• Carcinoma of urothelium • Transitional cell carcinoma are most common • Carcinoma *in situ* found in one-half of bladders with multiple papillary tumours [68]	• 75% are superficial at presentation • Metastasis to regional lymph nodes, bone, lung, other • 80% 5-year survival rate [68]	• Surgery can be curative • Chemotherapy, radiation, immunotherapy useful [119]	
Canine	• <2% of canine malignancies [25]	• Carcinoma of urothelium • Transitional cell carcinoma are most common [25]	• Most are invasive at diagnosis • Metastasis to regional lymph nodes, bone, lung, other • Demonstrated risk associated with obesity, insecticide and herbicide exposure [25,120] • Breed-associated risk [25]	• Surgery not effective • Modest sensitivity to platinum and doxorubicin chemotherapy • Sensitive to immunotherapy (BCG) and cox-2 inhibition	• Carboplatin [121] • Piroxicam single agent; combined therapy with cisplatin [122–124] • Photodynamic therapy [125]
Murine	• R26Cre-ERT [126]	• Invasive bladder carcinoma [126]	• Mice with human oestrogen receptor under control of ROSA26 locus R26Cre-ERT • Multifocal somatic mutagenesis [126]		

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Table 1 (*continued*)

Bladder cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
	• UPII/SV40T [127]	• Carcinoma *in situ* (CIS) • Moderate/high-grade carcinoma [127]	• Express SV40 large T antigen in the urothelium using the uroplakin gene promoter • High copy number of SV40: develop invasive/metastatic cancer; death at 3–5 months • Low copy number of SV40: develop bladder CIS at early age; subset develop papillary tumours at 10–16 months • 100% of CIS phenotype [127]		
	• CK19-Tag [128]	• Highly invasive, resemble invasive human bladder TCC [128]	• Develop CIS, stromal invasion, muscle invasion • 20% of affected mice develop lung metastasis • Death at 3 months [128]		
Pancreatic cancer					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 8.6/100 000 [68]	• Adenocarcinoma • Collagenous stroma, atrophic acini • Arise from exocrine pancreas most commonly [68]	• 4% survival at 5 years [68] • K-*ras* mutations common	• Surgery combined with 5-fluorouracil-based chemotherapy and radiation therapy	
Canine	• Rare [25]	• Adenocarcinoma—ductal or acinar [25]	• Metastasis at diagnosis most common [25]	• Palliative GI bypass for obstruction	
Murine	• RIP1-Tag2 [6]	• Late-stage pancreatic islet-cell carcinoma [6]	• Hyperplastic islets approx. 1 month • Angiogenic islets approx. 2.5 months • Solid tumours at 3 months • Die of tumour-induced hypoglycaemia or tumour progression at 3.5 months [6]		• Angiogenesis inhibitors [12] • Matrix metalloproteinase inhibitors [129] • Metronomic cyclophosphamide [130]

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Osteosarcoma					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 1000 new cases/year [131]	• High grade [131] • Complex karyotype with no consistent translocation	• Primary bone tumour most commonly appendicular • Aggressive metastatic phenotype • Metastases to lungs most common • 70% survival at 5 years with chemotherapy [131]	• Limb spare or resection of primary tumour • Adjuvant: doxorubicin, alkylating agents, platinum	
Canine	• 6000 new cases/year	• High grade [131] • Complex karyotype with no consistent translocation	• Primary bone tumour most commonly appendicular • Aggressive metastatic phenotype • Metastases to lungs most common • 60% survival at 1 year with chemotherapy • Occurs in older dogs • More rapid disease course than in humans [59]	• Amputation or limb spare of primary tumour • Adjuvant therapy: doxorubicin, platinum	• Limb-spare operative techniques [132] • L-MTP-PE[h] alone or in combination with chemotherapy [133,134] • IL-2 and chemotherapy inhalation [36,41] • IL-2 gene therapy [25] • Carboplatin [135] • Sustained-release local platinum delivery (OPLA-Pt) [136] • STEALTH liposome-encapsulated cisplatin (SPI-77) [34] • IGF-I blockage (OncoLAR) plus chemotherapy [57] • Split tyrosine kinase inhibitor [32]
Murine	• p53−/− • p53+/−	• Subset develops high-grade mesechymal tumours of bone • Also develop lymphoma and soft tissue sarcoma [137]	• p53−/−develops tumours at 4.5 months • p53+/−has a delayed onset at 18 months [137] • Metastatic to the lung		• Chemoprevention • Dehydroepisandrosterone • Quercetin • D-limonene • All-*trans*-retinoic acid [138]
	• C57BL/6-TgN(Amy1TAg)501Knw [139]	• Develop SV40 Tag-induced metastatic osteosarcoma	• Contain a liver α-amylase promoted-SV40 Tag hybrid gene • Metastatic osteosarcomas [139]		
	• SV40 T antigen [140]	• Subset of mice develops osteogenic sarcomas • Poorly differentiated [140]	• *Drosophila* hsp7 promoter fused with SV40 early region • Tumours form at about 7–17 months [140]		

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Table 1 (*continued*)

Soft tissue sarcoma					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	• 8300 new cases/year	• Loosely associated sarcomas with several histological forms • Two broadly defined classes: 1: Translocation/simple karyotype (e.g. Ewing sarcoma, synovial sarcoma), or 2: No specific karyotype/complex karyotype (e.g., fibrosarcoma, malignant fibrous histiocytoma) • 50% occur in the extremities	• Diverse biology dependent on specific histology • Mutated c-*kit* in gastrointestinal stromal tumours • 70–80% 5-year survival rate with chemotherapy and surgery [68]	• Radical surgery • Variably chemotherapy sensitive based on specific histology	
Canine	• 1% of malignant tumours in dogs	• Loosely associated sarcomas with several histological forms (fibrosarcoma, leiomyosarcoma, synovial sarcoma, hemangiopericytoma, histiocytic sarcomas) [58]	• Diverse biology dependent on specific histology	• Radical surgery	• Hyperthermia combined with irradiation [141]
	• 35/100 000 [18]	• Translocation status not studied	• Mutated c-*kit* in gastrointestinal stromal tumours [59]	• Poorly chemotherapy sensitive	• OPLA-Pt polymer for sustained local release of cisplatin [142] • Split tyrosine kinase inhibitor [32]
Murine	• p53−/−	• In addition to soft tissue sarcoma, develops lymphoma, haemangiosarcoma, and osteosarcoma [137]	• p53−/−develops tumours at 4.5 months		• See Osteosarcoma section for chemopreventative studies in p53 knockout
	• p53+/−		• p53+/−has a delayed onset at 18 months [137]		

(*continued on next page*)

Table 1 (*continued*)

Melanoma					
	Incidence or prevalence[a]	Histology	Biology	Treatment	Preclinical applications
Human	12.6/100 000 [18]	• Histological progression from benign to malignant lesions noted • Breslow depth of penetration predictive of prognosis [68]	• Cutaneous most common • Local at diagnosis most common • UV exposure established risk [143] • 80% 5-year survival for all stages [85]	• Surgical excision • IL-2 and IFN therapy [68]	
Canine (58)	• 25/100 000 (18)	• Histological progression of lesions not defined	• Buccal, ocular, and digital are malignant • 95% of cutaneous melanomas are benign • Not likely associated with sun exposure	• Surgery • Coarsely fractionated radiation therapy	• Tyrosinase DNA vaccine [144] • 'Bacterial superantigen' gene therapy (staphylococcal enterotoxin B) and either GM-CSF or IL-2 [39] • BCG [25]
Murine	• MT-HGF: metallothionine-hepatocyte growth factor [145]	• Nodular melanoma • Cells with spindle or epithelioid cytology [145]	• Develop melanoma after long latency • Metastases occur in subset of mice [145]		
	• Transgene B [146]	• Heavily pigmented melanocytic tumours • Large melanocytes [146]	• Tumour growth on ear • Significant portion die within 1 year [146] • Non-UV, non-carcinogen induced		
	• Hepatocyte growth factor/scatter factor (HGF/SF) [147]	• Melanocytes located in dermis, at epidermal/dermal junction, and basal layer of the epidermis • Histological progression similar to human [147]	• Neonatal UV irradiation of (HGF/SF) transgenic mice enhances tumorigenesis [147]		• (G. Merlino, personal communication)

[a] For all canine cancers, disease prevalence is provided [25].
[b] CHOP-like—refers to combination chemotherapy protocol including cyclophosphamide, doxorubicin, vincristine, and prednisone.
[c] Personal communication (Khanna *et al.*—Abstract: American Society of Clinical Oncology, 2002).
[d] AML, acute myelogenous leukaemia.
[e] CLL, chronic lymphocytic leukaemia.
[f] ALL, acute lymphocytic leukaemia.
[g] CML, chronic myelogenous leukaemia.
[h] L-MTP-PE (liposomal muramyl tripeptide phosphatidal ethanolamine): macrophage activator.
[i] EMICE: www.emice.gov—website for the Mouse Models of Human Cancer Consortium.

tumours are then used to derive cell lines or tissue fragments that can be injected or implanted, respectively, into large experimental groups of naïve mice that are syngeneic with the original GEM. Following transplantation, groups of mice can be expected to undergo synchronised tumour development and progression similar to that seen with other transplantation models. Brown and colleagues used leukaemia cells isolated from bone marrow, spleen or lymph nodes of PML-RARα mice as a source of tumour for injection into naïve FVB/N mice; transplanted leukaemic cells resulted in a disease course that was similar to that in the original PML-RARα mouse [15]. Experimental flexibility is created through the generation of transplantable reagents that can be used for the development of primary tumours, experimental metastases (i.e. tail vein injection) or spontaneous metastases (dissemination of metastases from a primary site). These models do not allow assessment of chemoprevention strategies but can be used to assess endpoints of tumour development, tumour progression and survival. Used in this way, GEM models continue to have the advantages of tumour growth in immune-competent hosts where tumour-microenvironment interactions are one species and syngeneic. This transplantation approach does limit the heterogeneity of the *de novo* tumours seen in the parent GEM. Therefore, therapeutic response or failure must be assessed in light of the 'selection' associated with the successful *in vivo* transplantation and passage of the tumour [16]. An additional caution is that the fidelity of a transplantation reagent (either cell line or tissue fragment) to the original GEM tumour phenotype is likely to be variable over time and over successive passages. Using the broadly defined experimental examples from Fig. 1 and included in Table 1, it is quite likely that most currently available GEM can be used in drug-development studies.

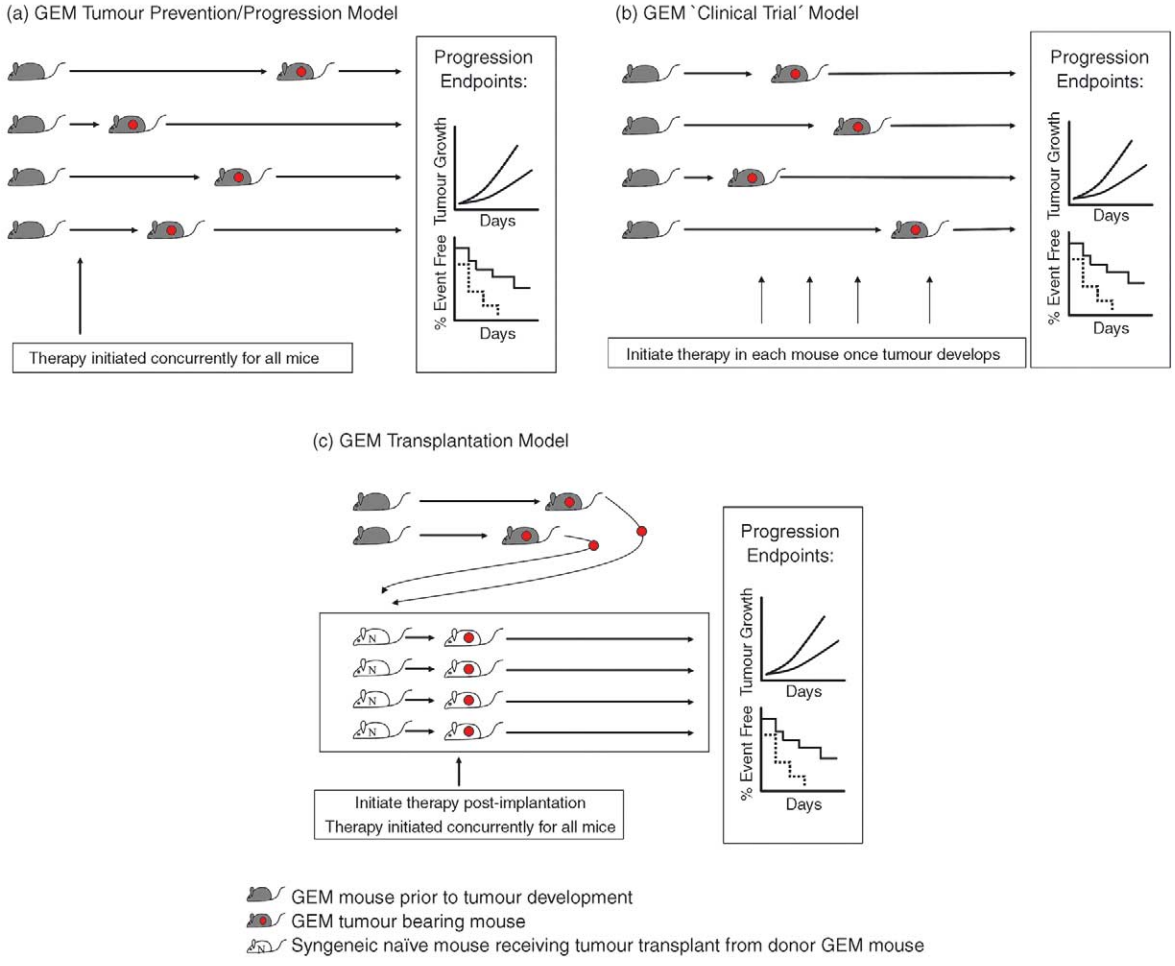

Fig. 1. Strategies for use of genetically engineered mouse models (GEM) of cancer in preclinical therapeutic studies. (A) In this experimental approach, mice receive concurrent administration of novel therapeutics before tumour development. Tumour development, growth and survival may be used as endpoints. (B) Mice are treated upon detection of a tumour/lesion. Tumours may be detected by physical examination or novel imaging strategies. Mice may then be randomised to receive the experimental drug or control. Each mouse is individually followed for endpoints of tumour progression. (C) Transplantation of GEM-derived tumours into naïve, syngeneic mice. In this approach, tumours that develop in GEM mice are resected and used as source tissue for transplantation into naïve mice. Groups of naïve mice that have received tumour transplants are then treated in a more conventional preclinical study design.

Results from these studies will determine if these mouse models can predict responses in human patients and if the predictions made will be superior to those associated with more conventional transplantation (xenograft) models of cancer.

Equally as important as the evaluation of anticancer agents' activity is the definition of pharmacodynamic endpoints or biomarkers of response for these novel agents. As novel therapeutics enter preclinical development it is essential that biomarkers be simultaneously developed and validated. GEM provide an unique opportunity to define simultaneously both the activity of novel therapeutic agents and the biomarkers that will be used to assess the activity of these agents when and if they reach the clinic. It is less likely that such validation studies will be possible through the use of more conventional transplantation models. The relevant carcinogenic progression associated with cancer-prone GEM and the species-concordant tumour-microenvironment interactions that are characterised by these models makes them ideal for the evaluation of biology-based therapies and their associated pharmacodynamic endpoints.

A potential obstacle to the use of GEM in drug development relates to patent rights in GEM. One family of patents concerns the OncoMouse technology, which are issued to Harvard University and exclusively licensed for most purposes to DuPont Inc. The most recent patent, issued in July 1999, will expire 17 years from the issue date. The patents are in force and effect in the US, Europe and Japan, but not in Canada. These patents claim rights in (i) mice or reagents developed from any non-human mammal containing any activated oncogene sequence; (ii) cell lines isolated from the above mouse model; and (iii) the use of such a mouse model for drug testing. DuPont has sought to enforce its licence rights under the patents by requesting that investigators' institutions enter into licensing agreements for non-commercial and commercial uses. The US Public Health Service (PHS) has entered into an agreement with DuPont that permits National Institutes of Health researchers to use GEM for non-commercial purposes and agrees to extend the same terms to institutions of PHS-funded investigators (for reference: http://ott.od.nih.gov/textonly/oncomous.htm).

DuPont has also executed agreements with some academic institutions, and with pharmaceutical and biotechnology companies. As stated above and relevant to this review article, these patents could specifically cover the use of an OncoMouse or derived tissue in preclinical drug development. The potentially restrictive effect of these patents on the use of GEM in drug development has been discussed extensively in the scientific and lay press. It is possible that the limited use of GEM by the pharmaceutical and biotechnology industry results from DuPont's exercise of its rights under these patents, which may include claims for 'reach through' rights to therapeutic agents discovered by the use of GEM.

3. Naturally occurring cancers in companion animals

A significant and at present under-utilised group of cancer models are the naturally occurring cancers seen in companion animals, primarily in pet dogs but also in cats. The significant anatomical and physiological similarities between dogs and man have been the basis for the use of dogs in biomedical research for over 70 years. Reports of the use of dogs in the field of cancer drug development extend to the first form of systemic chemotherapy, nitrogen mustard [17]. Dogs continue to be used to define the safety profiles for novel cancer agents destined for use in human phase I clinical studies. A current change of attitude seeks to include tumour-bearing pet dogs in the assessment of efficacy for new therapeutics.

Cancer in the companion animal (pet) population is a spontaneous disease. In many cases these spontaneous cancers share features with human cancers. Companion animal owners, motivated by prolonging the quality of their animals' lives, frequently seek out the specialised care and treatment of veterinary oncologists at private-referral veterinary hospitals and veterinary teaching hospitals. It has been estimated that there are approximately 55 million dogs and 60 million cats at risk for developing cancer in the USA [18]. Dogs are resistant to atherosclerosis-associated cardiovascular disease, so cancer is the principal cause of their death. In an autopsy series of 2000 dogs, 23% of all dogs, regardless of age, and 45% of dogs 10 years of age or older, died of cancer. Estimates of age-adjusted overall cancer incidence rates per 100 000 individuals/years at risk range from 243 to 381 for dogs and 156 to 264 for cats [19]. These rates are comparable to those reported by the National Cancer Institute SEER program for human beings (approximately 300 per 100 000) [17,19–21]. Incidence rates for certain malignancies in companion animals [e.g. canine osteosarcoma, non-Hodgkin's lymphoma (NHL)] are also higher than those observed in people. Crude estimates of cancer incidence suggest there are roughly 4 million new cancer diagnoses in dogs and a similar number in cats each year. The size of the group of companion animals with cancer should provide the opportunity to include them in studies of new cancer drugs.

Examples of such spontaneous models are listed in Table 1 and include NHL, prostate carcinoma, lung carcinoma, head-and-neck carcinoma, mammary carcinoma, melanoma, soft-tissue sarcoma and osteosarcoma. Many factors contribute to the value of these spontaneous cancers as relevant models for human cancer. These animals share many environmental risk factors with their human owners, suggesting their value

as sentinels of disease [22]. The strong genetic similarities between dogs and man have meant that dogs with spontaneous cancers can be used in the identification of cancer-associated genes [23,24]. These cancers share aspects of tumour biology and behaviour with human cancers, and in some cases have identical histological appearances and response rates to conventional chemotherapies [25]. In most cases the prevalence of these cancers is sufficient for preclinical trials and biological studies. The size of dogs and cats makes multimodality protocols feasible. Furthermore, the lack of 'gold standard' treatments permits early and humane testing of novel therapies, and the rapid progression and early metastatic failure seen in pet dogs permit the timely completion of clinical trials. Although cats with naturally occurring disease have been included in the study of many aspects of biology, most prominently in the fields of endocrine disease and retroviral infection [26–28]; the following discussion will focus on the use of companion dogs in translational research and preclinical cancer drug development.

The genetic similarities between dogs and man have become increasingly clear with the continuing efforts to sequence the canine genome. A recent report by Kirkness *et al*. suggest greater homology between dogs and man 'by several measures' than between either species and the mouse [29]. This genetic similarity and the outbred nature of companion animals provide a strong rationale for the use of dogs in biomedical research and, more importantly, dogs with spontaneous disease (including cancer). The genetics of cancer in pet dogs has been investigated to a limited extent. For the more commonly studied canine cancers, strong similarities with the matching human cancers (e.g. canine osteosarcoma and canine NHL) have been shown [25]. With the sequencing of the canine genome and the availability of canine expression microarrays, this genetic characterisation will continue, allowing the similarities and differences between human and canine cancers to be defined [30,31]. It is expected that a commercially available, canine oligonucleotide microarray will be available by the end of 2004.

The biology of cancer in companion animals, as with human cancers, is dependent on the specific cancer. In general, for a given cancer in dogs, progression is slower than in murine cancers, but more rapid than for the same human cancer. Table 1 includes a list of naturally occurring canine cancers that have been used, or are amenable for use, in preclinical studies. A brief description of each model, its natural biology and metastatic behaviour is also included in this table.

It is not likely that all questions related to the development of a drug can be or should be asked of a single model. Based on an understanding of the characteristics of each model system (genetics and biology), appropriate questions can be asked of any model including those that are naturally occurring. The answers should be integrated with information gained from several model systems as a novel therapeutic agent is assessed in the preclinical setting. Table 1 lists tumour types by cancer type (i.e. tumour histology). As the appraisal of a genetic profile/signature for cancers in general proceeds, it will become more relevant for a model to fulfill parts of the genetic signature of a human cancer rather than merely to resemble a human cancer histologically. London and colleagues demonstrated significant activity for a small-molecule inhibitor of the split tyrosine kinase receptor family (i.e. c-*kit*, platelet-derived growth factor receptor, vascular endothelial growth factor receptor) in dogs with grossly measurable cancers [32]. The responding histological types included metastatic sarcomas, carcinomas (including breast carcinoma) and cutaneous mast-cell tumours. The earlier demonstration of the autocrine activation of c-*kit* in canine mast-cell tumours could have predicted the responses in this disease following exposure to agents that inhibit c-*kit* signalling. In subsequent work by the same group, dogs with mast-cell tumours have been used as a naturally occurring molecular model of aberrant growth-factor receptor signalling and, more specifically, aberrant c-*kit* signalling [33]. These studies in dogs with mast-cell tumours have aided the translational development of this class of agents independent of histology by defining important pharmacokinetic and pharmacodynamic relations that would be difficult to show in other preclinical models or in human clinical trials. Opportunities to use canine cancers as spontaneous 'molecular' models will continue to increase as the molecular profiles of these cancers become better defined.

As discussed above, the translation of novel targeted and biology-based therapies to the clinical arena will require more sophisticated preclinical models. The biotechnology and pharmaceutical industries have recognised this need and have initiated studies in companion animals to assist in drug development. The primary rationale for the use of these models in preclinical efficacy studies is the immune competence of the host, the relevant and species-concordant tumour-microenvironment interactions, tumour heterogeneity, the spontaneous development of tumours and, more importantly, the spontaneous development of resistance patterns within an individual animal. Studies in companion animals can allow serial biopsies from target and non-target lesions, and repeated collection of body fluids (serum, whole blood, urine) from the same animal during exposure to an untried agent. With advances in the availability of dog-specific reagents, samples collected from treated animals can be used for gene-expression analysis, serum and tumour proteomics, single-cell sorting of circulating cells, as well as other biomarker analyses. Results from these studies will help in the design and success of similarly complex studies in human patients.

Companion dog studies may be informative at many points in the translational process. For agents that have not yet entered the clinic, companion dog studies may provide a combination of toxicity and efficacy data that support initiation of phase I studies in man [34].

For agents or classes that are currently in such phase I exploration, studies in dogs may define optimal treatment regimens, uncover uniquely sensitive histological types or validate biomarkers that will then be useful in phase II investigations [32]. The professional care, clinical pathology and diagnostic imaging required in canine work are similar in quality to those in human clinical trials, but are significantly cheaper. Well-designed, preclinical trials that involve and ultimately help companion animals are generally more acceptable to those concerned with animal rights than are laboratory animal studies. In the next section, examples of preclinical trials undertaken in companion animal cancer models are presented.

4. Preclinical studies involving companion animals

The size of companion animals, particularly dogs, has been of value in the assessment of several therapeutic methods that would have been difficult to translate from murine models alone. Such approaches have included surgery, radiation and hyperthermia, photodynamic therapy, inhalation therapy, gene therapy, and novel imaging [35–39]. Dogs with osteosarcoma have been used extensively in the development of preoperative and operative techniques to optimise the limb salvage now used in the management of childhood and adult bone tumours [40]. Similarly, dogs with soft-tissue sarcomas have been successfully used to define treatment protocols involving hyperthermia, radiation therapy and combinations of both with cytotoxic chemotherapy [34]. Similarities between dogs and man in respiratory anatomy, and in the relative size and distribution of primary lung cancer and cancers metastatic to the lung, have allowed the assessment of novel inhalation therapies [41]. Canine trials of inhaled interleukin 2 supported a successfully completed feasibility trial in patients with pulmonary metastases and follow-up trials with inhaled granulocyte colony-stimulating factor [42–44]. Trials of inhaled cytotoxic chemotherapy in dogs have also supported the development of this approach for human clinical trials [41]. Dogs have now been included in early trials of tumour gene therapy for locally accessible oral tumours and systemic metastatic disease [39,45]. The companion animal may be very important in studies that define the safety and efficacy of gene therapy approaches before their use in human patients. In all of these examples, the large size of dogs allowed specialised techniques or medical equipment to be evaluated and translated rapidly to human clinical trials.

Beyond the advantages of working with large animal models, provided by pet dog cancers, the similarities in tumour biology seen between specific human and canine cancers further contribute to their value as models of human cancer.

The value of histology specific studies involving spontaneous cancers in companion dogs was recognised over 30 years ago by Storb and colleagues [37]. Storb treated pet dogs with spontaneous lymphoma to optimise protocols for bone marrow transplantation and limit graft-versus-host disease [46]. Canine NHL is a useful naturally occurring model of NHL and other lymphoid malignancies in man (Table 1). Most canine NHL are described as high-grade B-cell cancers. At presentation, most dogs with NHL are asymptomatic and have generalised, non-painful enlargement of peripheral lymph nodes. This clinical presentation provides a relatively large window of opportunity to evaluate novel therapeutic agents before any conventional agents are administered. Conventional chemotherapy is likely to yield complete responses in over 80% of dogs with NHL; however the duration of this remission is short, with a median of 6–11 months [25]. This short duration of remission provides an opportunity to define the activity of novel therapeutic agents in the setting of minimal residual disease. Dogs with NHL are being used by many groups to assess the activity of novel cancer agents alone and in combination with conventional therapy. These combination trials are likely to be informative as they closely model the future clinical use of most novel cancer drugs.

Dogs with osteosarcoma share many similarities with human childhood osteosarcoma, including the histology of the primary tumour, the micrometastatic disease that follows successful control of the primary tumour, a period of micrometastatic disease that follows successful control of the primary tumour, responsiveness to platinum and anthracycline chemotherapy, and progression to gross metastases to the lung. Dogs with osteosarcoma have been successfully used to assess the clinical activity of liposomal muramyl tripeptide phosphatidyl ethanolamine (LMTP-PE). LMTP-PE is a liposome-encapsulated, synthetic macrophage activator based on the structure of the mycobacterial cell wall. Preclinical studies in murine cancer models suggested that LMTP-PE might be used in the management of metastatic disease [47–49]. The translation of these preclinical data to human clinical trials was greatly enhanced by studies in companion dogs with osteosarcoma [50–53]. These studies led to a recently completed multicentre, phase III, intergroup study by the Children's Oncology Group using LMTP-PE in child patients. The results of this study have not been formally reported, but preliminary analysis suggests a modest advantage associated with LMTP-PE treatment (R. Gorlick, personal communication). Interestingly, this benefit was seen only when

LMTP-PE was combined with ifosfamide chemotherapy. Studies are now under way to define a mechanism for the unexpected cooperation between ifosfamide and LMTP-PE. It is possible that LMTP-PE or biological agents that are responsible for its activity will return to dog trials before follow-up clinical trials in child patients are considered.

Dogs are the only species other than man that develop prostate cancer frequently. Most such cancers occur in older, sexually intact and castrated dogs. At diagnosis, canine prostate cancers are invasive carcinomas that do not respond to androgen ablation [25]. Their biology is associated with an aggressive metastatic phenotype, including metastases to regional lymph nodes, bone and lung. Although high-grade prostate intraepithelial neoplasia has been described in dogs, the relation of such lesions to invasive carcinoma is not well defined [54]. In addition, there is some evidence that the cell of origin for prostate cancers in dogs, unlike in man, is a ductal epithelial cell [56]. In many ways, companion dogs form a valuable model to answer questions about the biology and therapy of prostate cancer. Waters and colleagues have used companion dogs as a naturally occurring model of prostate carcinogenesis to define the role of selenium as a chemopreventive agent [56]. In this chemoprevention clinical trial, elderly dogs received either a high-selenium or a normal diet for 7 months. Dogs fed selenium-supplemented diets showed less DNA damage than control-fed dogs, suggesting a role for selenium in the prevention of prostatic DNA damage. The lifespan of dogs will allow chemoprevention studies to define the benefit of interventions such as selenium in a much shorter time than similar human studies. As suggested by Waters, the companion dog may also be helpful in validating credible surrogate markers for future chemoprevention trials. The use of companion dogs with prostate cancer in chemoprevention and therapeutic trials will be enhanced through the development of serum markers or profiles that can be used to define the presence of this relatively rare cancer of dogs.

Companion animal models cannot be used in all translational settings. The progression of cancer in dogs, as discussed above, is slower than in most murine models, and, although the prevalence of the disease is sufficient to make clinical trials worthwhile, it will take time for large-animal trials to provide informative data. A randomised clinical trial of a new agent in a companion animal cancer may require 1–3 years for completion [57]. A similar trial in a human population may require 5–15 years. With the development and validation of surrogate markers of response through companion animal trials, it is expected that information will be extracted from these trials in much shorter periods of time. The cost of clinical trials in companion animals is significantly greater than for most murine studies. In the development of new drugs, these costs are primarily associated with production of sufficient quantities of drug to treat large animals. The costs of implementing a preclinical study in companion animals can vary significantly, based on the design of the study and its endpoints. Recently, there has been progress in the availability of reagents to evaluate and characterise companion animal cancers, but availability continues to be a problem. Interestingly, many human antibodies, specifically those that detect phosphorylated proteins, cross-react with canine tissues. An important advance in our ability to characterise companion animal models has come from the canine genome project [58]. Efforts to validate reagents and further characterise models are ongoing in several laboratories around the world. Contributing to this effort, the intramural programme of the National Cancer Institute's Center for Cancer Research has recently launched the Comparative Oncology Program. Its goals will be to facilitate the use of companion animal cancers in cancer research through the characterisation of these models and the design and implementation of preclinical translational trials (http://ccr.nci.nih.gov/resources/cop/). The similarities and differences that are defined between these models and human cancers will be equally informative.

The evaluation of novel therapeutic agents in spontaneous cancer models, including GEM or companion animals, is becoming more common. In the very near future, results from these preclinical studies and from human clinical trials based on them will become available. With this information it will be possible to define which translational questions are best asked of these more sophisticated, although time-consuming and expensive, animal models. It is likely that spontaneous cancer models will fill the important gap between simple *in vivo* tumour models and human clinical trials.

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