ILAR Journal, 2018, 1–16

doi: 10.1093/ilar/ily014
Review Article

Of Mice, Dogs, Pigs, and Men: Choosing the Appropriate Model for Immuno-Oncology Research

Nana H. Overgaard[1], Timothy M. Fan[2], Kyle M. Schachtschneider[3], Daniel R. Principe[4], Lawrence B. Schook[3,5], and Gregers Jungersen[6]

[1]Department of Micro- and Nanotechnology, Technical University of Denmark, Kgs Lyngby, Denmark, [2]Department of Veterinary Clinical Medicine, University of Illinois, Urbana-Champaign, Illinois, [3]Department of Radiology, University of Illinois, Chicago, Illinois, [4]Medical Scientist Training Program, University of Illinois College of Medicine, Chicago, Illinois, [5]Department of Animal Sciences, University of Illinois, Urbana-Champaign, Illinois, and [6]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark

Address correspondence and reprint requests to Gregers Jungersen, Technical University of Denmark, Kemitorvet Building 204, Room 166 2800 Kgs. Lyngby, Denmark. E-mail: grju@dtu.dk.

Abstract

The immune system plays dual roles in response to cancer. The host immune system protects against tumor formation via immunosurveillance; however, recognition of the tumor by immune cells also induces sculpting mechanisms leading to a Darwinian selection of tumor cell variants with reduced immunogenicity. Cancer immunoediting is the concept used to describe the complex interplay between tumor cells and the immune system. This concept, commonly referred to as the three E's, is encompassed by 3 distinct phases of elimination, equilibrium, and escape. Despite impressive results in the clinic, cancer immunotherapy still has room for improvement as many patients remain unresponsive to therapy. Moreover, many of the preclinical results obtained in the widely used mouse models of cancer are lost in translation to human patients.

To improve the success rate of immuno-oncology research and preclinical testing of immune-based anticancer therapies, using alternative animal models more closely related to humans is a promising approach. Here, we describe 2 of the major alternative model systems: canine (spontaneous) and porcine (experimental) cancer models. Although dogs display a high rate of spontaneous tumor formation, an increased number of genetically modified porcine models exist. We suggest that the optimal immuno-oncology model may depend on the stage of cancer immunoediting in question. In particular, the spontaneous canine tumor models provide a unique platform for evaluating therapies aimed at the escape phase of cancer, while genetically engineered swine allow for elucidation of tumor-immune cell interactions especially during the phases of elimination and equilibrium.

Key words: cancer immunoediting; canine cancer models; comparative oncology; immunotherapy; porcine cancer models; translational immunology

Introduction

Cancer has recently surpassed cardiovascular diseases as the leading cause of death worldwide.[1] The increasing cancer incidence combined with the emergence of improved therapeutic strategies has driven research into fields such as how the immune system influences cancer development and progression. The term immunosurveillance has traditionally been used to describe how the immune system can protect the host from tumor development.[2] However, because immunocompetent individuals still develop tumors, the hypothesis of immunosurveillance being a

fully protective mechanism is challenged.[3] It has become well-recognized that the interplay between tumor cells and the immune system is extremely complex, and the ability of tumor cells to avoid immune destruction has been included as an official hallmark of cancer.[4] Cancer immunoediting describes a complex interplay in which the immune system not only protects against cancer but also induces tumor-sculpting mechanisms leading to reduced immunogenicity of tumor cell variants.[5,6] The concept of cancer immunoediting is composed of 3 phases: elimination, equilibrium, and escape[7,8] (Table 1). The kinetics by which each of the 3 cancer immunoediting steps occurs is speculated to differ between tumors, with aggressive tumors accelerating faster through these phases.[8,9]

The elimination phase encompasses the original concept of immunosurveillance, where the innate and adaptive immune systems collaborate to destroy the developing tumor.[6,10] Although more work is needed to fully elucidate the mechanisms behind this antitumor immunity, it is known to be partly mediated by release of cytotoxic granules from $CD8^+$ T cells and Natural Killer (NK) cells in addition to cytokine release from $CD4^+$ T cells and Natural Killer T (NKT) cells[11] (Table 1). A more detailed mechanism behind the elimination phase has been proposed by Dunn et al (2002).[6] In brief, the tumor becomes invasive when reaching a size that requires a distinct blood supply controlled in part by the production of angiogenic proteins.[12] Such invasive growth results in small disruptions in the adjacent tissue, thereby inducing inflammation, which leads to intratumoral infiltration of innate immune cells like dendritic cells (DCs), NK cells, NKT cells, γδ T cells, and macrophages. Upon recognition of tumor cells, these innate immune subsets produce interferon (IFN)-γ, which can induce tumor cell death by antiproliferative and apoptotic mechanisms. Moreover, these innate immune cells produce chemokines with the capacity to limit blood vessel formation. Tumor cell debris is then taken up by DCs, which migrate to the draining lymph node and induce tumor-specific $CD4^+$ T helper cells and tumor-specific $CD8^+$ T cells. Finally, these activated T cells home to the tumor, where the $CD8^+$ T cells in particular mediate antitumor activities.[6] If the immune system succeeds in completing this phase, the host is cleared of cancer with no clinical symptoms or progression to the additional editing stages[6,10] (Table 1).

However, as well as protecting the host, antitumor immunity can also induce tumor-sculpting mechanisms resulting in tumor editing.[5,8,13,14] Consequently, tumor cell variants with increased capacity to avoid immune recognition can develop, thereby entering the equilibrium phase (Table 1). This is a dynamic equilibrium that can last for several years and is believed to be the longest of the 3 phases.[6,8,15] Several underlying molecular mechanisms at the genetic and epigenetic level have been suggested to contribute to reduced immunogenicity of cancer cells during the equilibrium phase. In particular, increased genetic instability, reduced Major Histocompatibility Complex (MHC) class I expression, and defective antigen processing have been implicated in reducing tumor immunogenicity and facilitating tumor escape.[8,10,16–23] Enhanced secretion of immunosuppressive cytokines by tumor cells, increased induction of regulatory T cells, and tumor insensitivity towards IFN-γ have also been reported as important factors[24–27] (Table 1).

After a prolonged suboptimal immune response, selected tumor cell variants with reduced immunogenicity can become insensitive to immune recognition resulting in uncontrolled tumor growth. This is referred to as the escape phase,[6–8,28] and the tumor is now capable of proliferating in a fully immunocompetent host environment (Table 1), although the degree of immune cell infiltration still affects the prognosis of the patient.[29–31] Additional work is required to fully understand the complex interplay between cancer and the immune system, highlighting the need for animal models appropriately mimicking the human situation. Different animal models can provide unique insights into the distinct immunoediting stages (elimination, equilibrium, and escape) of cancer progression and empower cancer researchers to rationally combine various modeling systems necessary to generate high-value and translationally relevant immunobiologic data from future research investigations.

Table 1 Common Immunological, Tumoral, and Clinical Characteristics of Cancer Immunoediting

Phase	Immunological Characteristics	Tumor Characteristics	Clinical Characteristics
Elimination	Active immunosurveillance. Initial infiltration of tumors with DCs, NK cells, NKT cells, γδ T cells, and macrophages. Production of IFN-γ and chemokines. Recruitment of adaptive immune cells followed by antitumor reactivity mediated by $CD8^+$ T cells, NK cells, $CD4^+$ T cells, and NKT cells.	High expression level of MHC class I, efficient antigen processing, and presentation of tumor antigens to T cells. Production of angiogenic proteins, tissue disruption, and induction of inflammation.	No clinical symptoms. Potentially full regression of the developing tumor.
Equilibrium	Dynamic equilibrium between the tumor and the immune system. Anti-tumor immunity remains present.	Expansion of tumor cell variants with reduced immunogenicity. Lowered MHC class I expression and increased genetic instability and avoidance of immune recognition. Enhanced secretion of immunosuppressive cytokines. Increased induction of Tregs and insensitivity towards IFN-γ.	The longest of the three phases, which may last for several years.
Escape	Suppression of antitumor immunity and/or lack of recognition. T cells impaired by inhibitory cytokines and checkpoint molecules, limitations in nutrient availability, metabolic competition, reduction of oxygen levels, and increase in lactate production by the tumor cells.	Defective antigen processing and reduced antigen presentation to T cells. Insensitivity to immune recognition. Immunosuppressive tumor microenvironment.	Uncontrolled tumor growth in an immunocompetent host.

References.[6–11,13–20,22,24–28]

Abbreviations: DC, dendritic cell; NK cell, natural killer cell; NKT cell, natural killer T cell; MHC, Major Histocompatibility Complex; Treg, regulatory T cell.

Mouse Models of Immuno-Oncology

Syngeneic Mouse Models

For many years, mice have been the most commonly used animal model for immunological research and have provided a crucial elucidation of complex immunological pathways.[32–35] This in part reflects mice displaying reduced genetic variability, short generation intervals, easy maintenance, and the large number of commercially available reagents.[32,36] In cancer immunology, the most widely used mouse models involve inoculation of histocompatible (syngeneic) tumor cell lines into recipient mice, often of C57/BL6 or BALB/c background.[34,37,38] These syngeneic tumor models offer several advantages including reproducible tumor growth and simplicity in measuring tumor development over time, especially if the tumor cells are inoculated subcutaneously.[33,34,39] However, the off-site (heterotopic) injection of tumor cells in the subcutaneous tissues largely fails to recapitulate the normal microenvironment in which most tumor cells develop, and hence the operative mechanisms of immunosurveillance are likewise artificial. Additionally, the tumor cell lines tend to grow aggressively post injection, which causes studies to be terminated within a relatively short time due to ethical considerations and temporally constrains the time allowed for trafficking of immune cells and the natural development of antitumor immunity. Furthermore, the tumor cell lines differ in their intrinsic immunogenicity; therefore, the resulting tumor microenvironment often does not represent what is seen in human patients.[40,41]

Orthotopic implantation is administration of a given tumor cell line into the relevant tissue for that specific tumor. In contrast to subcutaneous injection, orthotopic implantation has been shown to better recapitulate the tumor biology, tumor environment, and disease progression.[42] In particular, the early steps of metastasis and angiogenesis have been modelled more appropriately using orthotopically implanted tumors.[42–45] Moreover, orthotopically implanted tumors have provided a valuable system for evaluation and understanding of checkpoint inhibition in various preclinical cancer models.[46–48] To date, several types of orthotopically implanted tumor models have been established amongst others, including transplantation in the brain (GL261 cells),[49] the mammary fat pad (4T1 and EMT6 cells),[50,51] intrasplenic (Panc02 cells),[52,53] and in the bladder (MBT-2 cells).[54] Overall, these models may serve as more clinically relevant systems, although the technicality of transplanting the tumor cells is more complex and labor-intensive compared to subcutaneous administration.[42]

Genetically Engineered Mouse Models

Although syngeneic mouse models are immunocompetent, they do not offer the opportunity for directly testing human targets. For this reason, syngeneic models are increasingly replaced by genetically engineered mouse (GEM) models, human xenograft, and patient-derived xenograft models.[39] An almost unlimited number of GEM models exist, with those for cancer research purposes typically produced through deletion, mutation, or overexpression of genes known to be crucial for cellular transformation and malignancy.[55] GEM models are very useful for studying the effect of specific mutations on tumor progression in an immunocompetent host.[55–58] By changing the genetic profile of these mice, it is possible to introduce mutations resulting in conditional expression/overexpression or loss/gain of function of genes known to be involved in transformation and tumorigenesis.[55,58] Moreover, tissue-/organ-specific targeting of the mutation or targeting to specific developmental stages during disease progression are valuable research tools for understanding the complex mechanisms underlying transformation and malignancy.[55,59]

Despite this, GEM models often fail in mimicking the complexity of human tumors that are often driven by stochastic genomic instability.[55] Some mouse models of cancer appear to be driven by homozygous mutations, whereas human cancers are most likely heterozygous with a functional wild-type allele. As such, the knockout of specific genes or pathways in GEM models may fail to recapitulate the chaotic manner in which malignant transformation occurs during spontaneous tumor development in human cancer patients. Although no ideal animal model can fully recapitulate the stochastic nature of human tumorigenesis, certain strategies have been developed to generate GEM models with more heterogeneous tumors of clinical relevance. Such approaches include, for instance, single-cell knockouts to achieve sporadic loss of gene expression and subsequently in vivo mosaics[59,60] as well as chemical- or UV-induced models, which can result in heterogeneous tumors arising from a multistep process.[61,62]

Xenograft Models and Humanized Mice

Xenograft models, which involve the transplantation of human cancer cell lines, or patient-derived tumor cells in the case of patient-derived xenograft models, into immunodeficient mice represent another commonly used mouse model for cancer research.[63–65] These models offer a unique tool for testing anticancer drugs targeting human proteins in mutated cancer as well as individualized and patient-specific treatments.[55] Moreover, engraftment of surgically resected tumor biopsies into these immunodeficient mice allows for an in vivo system, where interactions between, for instance, tumor cells and stromal cells can be evaluated.[65] Xenograft models undeniably add valuable knowledge to the research field; however, they are fairly expensive and labor intensive.[66,67] Also, the arising tumor is not exposed to any immune-mediated pressure due to the lack of an endogenous immune system.

To address the limitations associated with using an immunodeficient host, humanized mice have been developed. These mice are either genetically engineered to carry human genes[57] or developed through engraftment of human immune cells into an immunodeficient host.[68–71] Notably, humanized mice have provided an important tool for obtaining knowledge within the field of checkpoint inhibitors targeting, for instance, cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), programmed cell death-1 (PD-1), and programmed death ligand-1 (PD-L1).[72] Moreover, therapies combining chimeric antigen receptor (CAR) T-cell therapies with checkpoint inhibition have been tested in humanized mice.[73,74] Despite this, humanized mice are often on the *Il2rg*$^{-/-}$ background; they lack both lymph nodes and Peyer's patches,[75–77] which are major secondary lymphoid organs necessary for mature DCs to interact and potentially activate naïve T- and B-lymphocytes. As such, humanized mice are devoid of key organized immune microenvironments critical to initiating robust immune responses. Furthermore, humanized mice are challenged in their capacity to restore MHC class I and II-selecting elements, which are crucial for shaping the T-cell repertoire.[78]

It is becoming increasingly recognized that mice often poorly mimic human diseases, even when sophisticatedly manipulated with genetic techniques.[79,80] An ideal animal model for cancer research should preferably be fully immunocompetent to properly mimic human immune responses.[39,81] Although some mouse

models are immunocompetent, they often still display a very narrow MHC class I representation due to inbreeding. Consequently, this might result in unrepresentative results when compared to outbred animals and humans.[32] Overall, no perfect animal model capable of fully recapitulating the complexity of human disease exists. Mouse models have indeed provided the field of immuno-oncology with invaluable insight, but there remains a need for large animal models encompassing a fully competent immune system, which may function as a link between murine studies and the clinic. Given their comparable body size and metabolic physiology to human beings, as well as their well-annotated genomes, canine and porcine models of human cancer are uniquely situated to serve as excellent comparative tumor models.

Canine Models Of Immuno-Oncology

Cancer in pet dogs is common and has been reported as a leading cause of death in aging dogs, accounting for greater than 1 in 4 deaths.[82,83] As cancer in dogs occurs spontaneously and displays similar characteristics to many specific human tumor histologies, canine models are becoming more widely used in preclinical cancer research.[84–86] Representative of this research opportunity, in 2003 the National Cancer Institute's (NCI) Center for Cancer Research established the Comparative Oncology Program to facilitate and support the design, sponsorship, and execution of translational trials in pet dogs to test novel anti-cancer drugs prior to human clinical trials.[87] There are several advantages unique to canine models that were recognized and leveraged to expedite novel drug development ultimately slated for human usage. Because dogs are companion animals, they often live together with humans; therefore, they are exposed to the same environmental risk factors and might to a certain extent have a diet similar to humans.[88,89] As with humans, a correlation between spontaneous tumor incidence and age is found in dogs.[90] Additionally, from an evolutionary point of view, dogs are more closely related to humans than mice[91,92] and share more similar physiologic and immunobiologic traits. Lastly, the high degree of homology in the human and canine genome makes analysis of DNA damage as well as epigenetic changes during tumor development and progression more facilely traceable and possible in outbred dogs.[91,93,94]

Recently, several canine tumor histologies have been intensely studied using molecular cytogenetic techniques such as comparative genomic hybridization, oligonucleotide arrays, fluorescence in situ hybridization, and gene expression profiling. Based upon these genomic investigations, several conserved genetic similarities have been identified between canine and human tumors, including DNA copy number variations, structural chromosome aberrations, and differential gene expression patterns.[95–107] These findings of shared genetic perturbations associated with distinct tumor histologies in both dogs and human beings further support the potential value of pet dogs with certain types of naturally occuring tumors as a unique model system for human-relevant cancer research. Importantly, canine tumors believed to be immunogenic including osteosarcoma, lymphoma, urothelial carcinoma, mammary gland carcinoma, melanoma, and brain cancers have been the primary focus of most genomic-based investigations.[95–97,99,100,102,104–107]

The Canine Immune System

The canine immune system demonstrates a close homology to the human counterpart,[108–110] and many of the same immune markers have been validated in the canine species. Because tumors in pet dogs arise in an immunocompetent host, canine models enable the design of experiments that elucidate the complex interplay between cancer cells and the immune system as well as the natural progression of malignant transformation under the evolutionary pressures exerted by host immunosurveillance. Using human antibodies toward T-cell markers, it is now possible to distinguish canine activated T cells and central memory T cells by flow cytometry,[110] thus providing an important tool for vaccine research purposes. Adding to the strength of dogs to cancer vaccine research is their recognized breed-specific restriction in MHC expressions,[111–113] thereby allowing cancer researchers to focus efforts on "high-value" neoantigen discovery most likely to elicit potent cytotoxic T-cell responses. Despite being limited in scope to date, some studies have evaluated tumor immune cell infiltrates in canine cancer models. Flow cytometric analysis has shown the presence of both $CD4^+$ and $CD8^+$ tumor infiltrating lymphocytes within canine mammary tumors.[114] Another study using dogs with metastatic lesions showed an increased CD4/CD8 T-cell ratio, which also correlated with decreased survival rate.[114] In studies of canine B cell lymphoma, a worse prognosis was found in dogs with increased representation of tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells,[115–117] and cytotoxic T-cell-mediated killing of autologous lymphoma cells has been demonstrated in vitro.[116] Collectively, these preclinical and clinical findings provide strong support for including the canine species as an immune competent model system for immuno-oncology research.

Immunotherapy Research Using Canine Models

Leveraging the immune system to fight cancer can take many different, yet synergistic, strategies that engage the cellular players comprising the innate and/or adaptive immune systems. Classically, innate immune cells including neutrophils, marcrophages, and NK cells can be activated through engagement of diverse cellular receptors with cognate ligands of exogenous (pathogen associated molecular patterns) or endogenous (alarmins) nature, while cells of the adaptive immune system including B and T lymphocytes can be activated by primed antigen presenting cells. In addition, eliciting adaptive antitumor immunity can be mediated by both active and passive immunotherapeutic interventions such as vaccines and monoclonal antibodies, respectively. As immunobiologic reagents and therapeutics have become more readily available, many of these different approaches for stimulating both innate and adaptive systems, either passively or actively, have been investigated in pet dogs with cancer and a nonexhaustive list of example strategies are summarized in Table 2, with some of the most recent strategies further described below.

For immunotherapy purposes, canine tumor models offer a very powerful research tool. As monoclonal antibodies blocking CTLA-4, PD-1, and PD-L1 have provided impressive results in the clinic, it is desirable to have a preclinical animal model expressing these molecules. CTLA-4, PD-1, and PD-L1 expression have all been shown in a variety of canine solid and hematopoietic tumors.[118–123] In fact, the PD-1/PD-L1 pathway in dogs is associated with T-cell exhaustion, as often reported for humans.[119] Due to limitations in commercially available canine reagents, detailed studies with checkpoint inhibitors in dogs remain preliminary in scope and nature; however, early evidence demonstrates that blockade of PD-1/PD-L1 can lead to enhanced T-cell proliferation and cytokine release.[120,122,123] Whether these

Table 2 Strategies for Stimulating the Innate and Adaptive Immune System in Pet Dog Cancer Models

Immune Arm	Immunotherapeutic Strategy	Specific Methodology	Tumor Type	Reference
Innate				
	Innate immune cell activation	Localized radiation and autologous NK cell intratumoral transfer	Osteosarcoma	229
	Modulation of immune signaling	Localized radiation, TLR activation, and indolamine-2,3-Dioxygenase inhibition	Melanoma, STS	230
	Macrophage activation	Liposome MTP-PE infusion	Osteosarcoma	200
Adaptive (passive)				
	Exogenous cytokine therapy	Intravenous liposome-DNA complexes with interleukin-2 gene	Osteosarcoma	231
		Inhalation therapy with liposome interleukin-2	Osteosarcoma	232
		Intralesional interleukin-2	Urothelial carcinoma	233
		Intratumoral interleukin-2	Transmissible venereal tumor	234
	Monoclonal antibody therapy	*Ex vivo* PD-L1 blockade to mitigate T cell exhaustion	Various solid tumors	119
		In vitro PD-1 blockade to induce TIL activation	STS, adenocarcinoma	122
		In vivo PD-L1 blockade in cancer-bearing dogs	Melanoma, STS	120
Adaptive (active)				
	Adoptive transfer of T cells	Autologous T cell transfer following cytokine activation	B cell lymphoma	235
		Autologous lymphokine-activated T cell transfer	Melanoma, others	236
	Genetically-modified T cells (CAR-T)	Generation of CAR-expressing T cells specific to HER2 epitope-in vitro	Osteosarcoma	237
		Generation of CAR-expressing T cells specific to CD20	B-cell lymphoma	127
	Vaccination	HER2-targeting Listeria monocytogenes vaccination	Osteosarcoma	139
		Adenovirus DNA-electro-gene-transfer targeting dog telomerase reverse transcriptase	B-cell lymphoma	238,239
		Lipoplexes with HSV-TK and canine INFβ; tumor extract vaccine + cytokines	Melanoma	240
		Xenogeneic human tyrosinase DNA vaccine	Melanoma	241

Abbreviations: CAR, chimeric antigen receptor; NK, natural killer; PD-1, Programmed cell death-1; PD-L1, Programmed death-ligand 1; STS, soft tissue sarcoma; TIL, tumor-infiltrating lymphocyte; TLR, Toll-like Receptor.

observed immunobiologic activities will be adequate to produce robust clinical benefit in a substantial fraction of treated pet dogs remains to be determined, yet early results indicate some measurable immunobiologic activity against specific solid tumors including oral melanoma and soft tissue sarcoma.[120]

Most recently, genetically engineering of CAR T cells has been heralded as an immunologic breakthrough for the management of pediatric acute lymphoblastic leukemia in human beings.[124,125] Although this genetic manipulation technology remains in its infancy for veterinary medicine, CAR T cells have shown promising results in dogs as a proof-of-concept for the management of both hematopoietic (B-cell lymphoma) and solid (osteosarcoma) tumors.[126,127] Therefore, pet dogs might in the future serve as an important model in elucidating the design of treatment regimens that maximize therapeutic benefit yet minimize adverse events often observed upon CAR T-cell therapy.[128]

The establishment of active adaptive immunotherapy through tumor vaccination strategies remains a priority in human cancer patients. Although preventative vaccines against hepatitis B virus and human papillomavirus have dramatically decreased the incidence of hepatocellular and cervical cancers, respectively,[129,130] the utility of therapeutic cancer vaccines remains limited. In 2010, the FDA approved sipuleucel-T (Provenge), a vaccine that utilizes tumor lysate-loaded dendritic cells to activate the immune system against castration-resistant prostate cancer,[131,132] and to date this remains the only approved therapeutic cancer vaccine in people. In terms of cancer vaccine trials in dogs, whole tumor cell lysate vaccines have been tested either as combination therapy or stand-alone treatment.[133–135] Most notably, in 2007, a xenogeneic DNA vaccine (Oncept) targeting the human tyrosinase protein was the first therapeutic vaccine to be approved for treatment of canine oral melanoma.[136,137] Although considered the first of its kind, the definitive immunostimulatory potential and clinically benefit derived from this xenogeneic DNA vaccine strategy would be substantially bolstered through the conductance of a large, prospective, randomized phase III clinical trial in pet dogs. In addition, canine vaccine trials targeting telomerase reverse transcriptase, heat-shock proteins, and the human vascular endothelial growth factor protein have been performed.[92,136,138] Notably, these trials all share the aim of treating cancer in dogs rather than using the canine tumor models as a link between rodent studies and human clinical trials. However, at least 2 examples exist that seek to leverage the pet dog as a comparative tumor model for the development of immunotherapeutic strategies to be employed in human cancer patients. First, a *Listeria monocytogenes* vaccine strategy has been evaluated in pet dogs with osteosarcoma, and initial results support the generation of a potent adaptive immune response translating into substantive improvements in overall survival time.[139] Second, a DC-based vaccine in combination with IFN-γ administration has been demonstrated to improve the clinical outcome in tumor-bearing dogs,

thereby supporting the use of canine models for preclinical testing of human anti-cancer therapies.[140]

Despite the many benefits of canine cancer models, their use for therapeutic cancer vaccine development has a number of important drawbacks. The low number of known canine tumor antigens,[138] the increasing ethical regulation of experiments on companion animals,[89] and the limited number of commercially available reagents undeniably make canine translational research more difficult.[90] Although dogs are more outbred than mice, modern dog breeds are the results of line inbreeding, thus questioning whether canine models can properly mimic human heterogeneity.[36] Therefore, although canine models provide some important advantages over murine models, there is still a need for alternative large animal cancer models, and the most robust investigations will likely be derived from the utilization of a panel of animal models.

Porcine Models of Immuno-Oncology

Pigs are valuable models for studying immune responses toward infections.[141–143] Moreover, porcine models are becoming increasingly used for human biomedical research and as unique research tools for surgical procedural training.[144–146] The advancement in using porcine models is due to the high degree of homology in anatomy, physiology, size, cell biology, key metabolizing enzymes, genetics, and epigenetics between pigs and humans.[147–157] In addition, the life-span of the pig also offers an opportunity to monitor and characterize disease development and progression over a human-relevant amount of time.[36,149,158] Importantly for cancer research, porcine somatic cells, consistent with human cells, suppress telomerase activity in most tissues, which is then reactivated during tumorigenesis.[159,160]

Although mice are closer to humans phylogenetically, pigs and humans share a higher similarity in protein structure.[161] A detailed comparison of immune-related genes across several species revealed that pigs are more closely related to humans at the immunome level than mice.[141] In addition, the number of species-unique immune-related genes is considerably lower in pigs than in mice.[141] Using orthology preservation analysis of the immunome, the authors found 188 genes shared across humans, mice, and pigs. When evaluating species-unique immune-related genes, humans and pigs showed 37 and 16 genes, respectively. In contrast, 174 genes relating to various immunological pathways were found to be present only in the mouse,[141] clearly indicating crucial differences in the immune system between rodents and larger animals, including pigs and humans. Recently, the same authors compared the inflammasome across humans, pigs, and mice. Here, they clearly showed a murine expansion in the number of 7 different pattern recognition receptors compared to the 2 other species analyzed.[161] For instance, mice displayed 57 different receptors belonging to the NK cell receptor subfamily of the C-type lectin superfamily, whereas only 24 and 23 were found in the human and porcine system, respectively.[161] As NK cells are crucial players of mediating antitumor immunity and limiting tumor metastasis,[162,163] such differences need to be taken into account when interpreting immuno-oncology research. Combined, these data support the notion that preclinical results obtained in porcine models have several advantages compared to rodent models.

The Porcine Immune System

Overall, the porcine immune system comprises the same immune cell populations as demonstrated in humans.[143,164] For instance, the porcine Treg population expresses markers similar to the human population, namely CD4, CD25, and FoxP3.[165,166] However, some important differences do exist between the porcine and the human immune system. Porcine peripheral blood comprises a large number of γδ T cells, representing up to 50% of the total blood lymphocyte population in young animals.[167] In contrast, the representation of γδ T cells in human peripheral blood sampled across the world is less than 10%.[168] Although the functional properties of γδ T cells are not fully understood, it is suggested that these cells display both cytolytic activity and capacity to perform antigen presentation.[165]

Another notable difference is that the porcine T-cell pool comprises a large proportion of $CD4^+$ T cells coexpressing the CD8α homodimer in peripheral tissues.[169,170] In pigs, these $CD4^+CD8\alpha^+$ T cells are defined as an activated/memory $CD4^+$ T-cell population recognizing antigens in the context of MHC class II.[165,171] As this $CD4^+$ T-cell population expresses the $CD8\alpha^+$ homodimer, expression of the CD8β molecule is commonly used to define porcine cytotoxic T cells.[164,165] In addition, the lymphocyte migration pattern differs slightly between pigs and humans due to the porcine lymph nodes being structurally inverted.[172] Consequently, porcine lymphocytes, similar to humans, enter the lymph node via L-selectin$^+$ high endothelial venules. However, porcine T and B cells leave the lymph node by directly entering the blood stream via high endothelial venules rather than migrating out via the efferent lymph as in humans.[172,173] Despite the increased representation of $CD4^+CD8\alpha^+$ T cells in porcine peripheral blood and the inverted lymph node morphology, there are currently no indications of these differences resulting in any significant functional differences between the human and porcine immune system.[173]

The porcine MHC molecule is commonly referred to as the swine leukocyte antigen (SLA). As pigs are largely outbred compared to rodents, fully immunocompetent porcine models display a high MHC class I allelic diversity with the number of known alleles continuously expanding with improved typing methods and growing interest in swine for biomedical research.[174,175] In particular, the development of a Next Generation Sequencing-based SLA-typing approach has allowed a fast identification of expressed SLA class I molecules,[174] thereby allowing selection of MHC-matched animals to be used for instance in a vaccine protocol or other immunological assays.

Immunotherapy Research Using Porcine Models

Although pigs have provided valuable findings for infectious diseases, porcine models have had limited use thus far in experimental oncology. The 2 most common cancer types found in pigs are lymphosarcoma and melanoma.[176] Porcine skin is very similar to human skin both in terms of morphology and functional characteristics,[177] providing a unique model for studying skin cancers like melanoma. For many years, the Sinclair minipig and the melanoblastoma-bearing Libechov minipig (MeLiM) model have been the 2 most commonly used porcine spontaneous melanoma models, although the underlying genetic changes resulting in the melanoma development are not well understood.[176,178] Despite this, a study in the MeLiM model has contributed to a better understanding of melanoma progression and identified RACK1 as a potential marker of malignancy in human melanoma.[179] In recent years, porcine severe combined immunodeficiency models have also been developed.[180–185] As in the rodent equivalents, porcine porcine severe combined immunodeficiency animals lack T and B cells, allowing them to be used for xenotransplantation studies including engraftment of human tumor and immune cells.

Genetically Engineered Porcine Models

To expand the use of pigs in experimental oncology, several genetically modified porcine models of human cancer have been developed. By overexpressing the human *GLI2* gene, it was possible to develop a model with basal cell carcinoma-like lesions.[186] In addition, colorectal cancer[187,188] and breast cancer[189,190] models have been developed, although these animals either lacked in vivo tumor development or displayed lethality issues. Modification of either the tumor suppressor gene *TP53* or the oncogene *KRAS* has enabled the development of porcine models giving rise to various cancer types. Mutational silencing of the *TP53* tumor suppressive pathway is observed in approximately 33% of human cancers.[191] Such mutations in the *TP53* gene are often associated with increased cell proliferation, survival, invasiveness, and metastasis.[192] The porcine models express the $TP53^{R167H}$ dominant negative mutation, which is equivalent to the frequently observed $TP53^{R175H}$ mutation in humans.[191,193] Upon expression of $TP53^{R167H}$, the pigs develop both lymphoma and osteogenic tumors.[194]

Furthermore, the *RAS* gene is mutated in approximately 25% of all human cancers, with *KRAS* being the most commonly mutated isoform.[191] The RAS protein is a GTPase driving cellular proliferation, and oncogenic RAS especially promotes progrowth, proangiogenic, and antiapoptotic signals.[195] Specifically for $KRAS^{G12D}$, this oncogenic activating mutation promotes metastasis in human pancreatic cancer in part by downregulating E-cadherin.[196] Although histopathology is yet to be determined, a porcine model with inducible $KRAS^{G12}$ has been developed.[194] Upon xenotransplantation, in vitro- transformed porcine mesenchymal stem cells expressing both the $TP53^{R167H}$ mutation and the $KRAS^{G12D}$ mutation have successfully established tumors in immunodeficient mice.[197] However, the only transgenic pig combining both the $TP53^{R167H}$ dominant negative mutation and the $KRAS^{G12D}$ oncogenic activating mutation is a model known as the Oncopig.[191] The expression of the 2 mutations is under control of a CAG promoter. Due to the internal ribosome entry site element, bicistronic expression of the mutated transgenes, $KRAS^{G12D}$ and $TP53^{R167H}$, is possible. Because every cell in the Oncopig has this expression construct, the model enables induction of a broad range of cancer types upon exposure to Cre recombinase.[191]

In vivo induction of sarcomas with regional leiomyosarcomas has been shown upon intramuscular, testicular, and subcutaneous injection of adenoviral vectors encoding Cre recombinase into Oncopigs.[191] Successful in vitro transformation of 11 different Oncopig cell lines has been established, as described in detail elsewhere.[36] Although limited in scope, some immunological characterization of the Oncopig intratumoral landscape has been performed. Using immunohistochemistry, infiltration of $CD3^+$ cells was shown in Oncopig hepatocellular carcinoma.[198] A more detailed and T-cell-focused evaluation of the immunological landscape in Oncopig sarcomas was recently performed, where pronounced T-cell infiltration to the tumor site was demonstrated (Overgaard et al, 2018, submitted). The tumor microenvironment was especially enriched with cytotoxic and activated immune cells. This, in conjunction with RNA-seq analysis revealing elevated gene expression of the immunosuppressive molecules *CTLA4*, *PDL1*, and *indoleamine 2,3-dioxygenase 1* in tumor tissue, supports the use of this transgenic porcine model for evaluation of the complex interplay between the tumor and the immune system of the host.

Ongoing and Future Translational Opportunities

Efforts are made to promote a One Health approach to evaluate new treatment options for cancer in canine animal models through the Comparative Oncology Trials Consortium at NCI as a major clinical trial hub across Northern America (United States and Canada). Further, a group of Academic Veterinary Teaching Hospitals in the United States/Canada recently established the Comparative Brain Tumor Consortium to improve the knowledge, development of, and access to naturally occurring canine brain cancers, specifically glioma, as a model for human disease.[199] Supporting the merits for the NCI's (Comparative Oncology Trials Consortium and Comparative Brain Tumor Consortium) translational efforts, existing evidence for the value of pet dogs with cancer in expediting anticancer drug development are multiple. Perhaps the best example for pet dogs to be included in the new drug or biological agent development path is mifamurtide, which is liposome encapsulated MTP-PE.[200] Although the data packet for mifamurtide was deemed insufficient for FDA approval, the European Medicines Agency was convinced of mifamurtide's activity and in 2004 approved its use for the treatment of high-grade, nonmetastatic, resectable osteosarcoma in human beings. In addition to mifamurtide, other investigational agents that included pet dogs with cancer in the pathway towards investigational new drug designation and human Phase I clinical trials include GS-9219, KPT-335, and PAC-1.[107,201–205]

Given the immune competency of pet dogs with cancer, and underscoring the unique and valuable potential of large animal models in cancer research, the NCI recently launched a request for proposals to support canine clinical studies evaluating the feasibility and activity of immunotherapeutic agents and novel drug combinations such as immune modulators, molecular targeted agents, chemotherapy, and/or radiation.[206] Clinical studies will be accompanied by laboratory correlative studies that seek to describe, characterize, and understand the cellular and molecular mechanisms that determine the antitumor response (or lack of response) in dogs with spontaneous tumors. Specifically, the spontaneous tumor types that have been deliberately targeted as comparative for immunotherapeutic development include lymphoma,[92,98,207,208] osteosarcoma,[95,97,209–212] mammary gland cancer,[106,107,213,214] brain cancer,[199,215–217] melanoma,[218–220] and transitional cell carcinoma[221,222] (Table 3).

Complementing spontaneous tumor models in pet dogs, the development of genetically modified pigs has allowed for several tumor types to be studied in these large experimental animal models. In particular, basal cell carcinoma,[186] colorectal cancer,[187] breast cancer,[189,190] soft-tissue sarcoma,[191,223] hepatocellular carcinoma,[198] pancreatic ductal adenocarcinoma (Principt et al., 2018, submitted), lymphoma,[193] and osteosarcoma[193,197] (Table 3) are among the tumor types that are currently in focus. However and as previously mentioned, both the colorectal cancer[187,188] and breast cancer[189,190] models currently either lack in vivo tumor development or display issues with lethality. Although there are obvious ethical problems in development of genetically modified pet animals for cancer studies, several genetically modified swine have already been developed to study cancer development as outlined above. With the emergence of precision gene editing tools, such as CRISPR/Cas9 or TALEN technologies, the potential for development of point-mutation models as well as single and multiplexed recombinants using homology-directed repair is a real and accessible option for development of new complex cancer models as well as complex comorbidity models.[149]

Table 3 Cancer Types Mimicked Either by Spontaneous Canine Models or Genetically Engineered Porcine Models

Spontaneous Canine Tumor Models (NCI Recognized)[167]	References	Genetically Engineered Porcine Tumor Models	References
Lymphoma	72,168–170	Lymphoma	193
Osteosarcoma	171–176	Osteosarcoma	193,197
Mammary gland cancer	177–180	Breast cancer	189,190
Brain cancer	166,181–183	Soft-tissue sarcoma	191,223
Melanoma	184–186	Hepatocellular carcinoma	198
Transitional cell carcinoma	187,188	Pancreatic ductal adenocarcinoma	Principe et al., 2018, submitted
		Basal cell carcinoma	186
		Colorectal cancer	187

Abbreviations: NCI, National Cancer Institute.

Because cancer is not one disease and different tumor types require specific treatment strategies,[224] a "one size fits all" universal animal model for preclinical testing or studying the complex pathways of tumor/immune cell interactions does not seem realistic. With the concept of cancer immunoediting in mind, it could be suggested that different large animal models should be used for evaluating the different phases of cancer immunoediting. For instance, and although complete histological regression of human melanoma lesions is a rare occurrence limited to relatively few case studies,[225] melanoma remains one of the human tumor types most commonly displaying spontaneous regression.[226] Interestingly, lesions of porcine melanoma models display a high tendency of spontaneous regression, with the MeLiM model showing complete clearance in up to 96% of the cases.[227,228] From this, it could be speculated that porcine models with their apparant efficient antitumor immunity provide a unique model for studying both the elimination and equilibrium phases of cancer. In contrast, the spontaneous canine tumor models with well-established, long-term tumors provide a platform for studying and testing immunotherapeutic agents aimed at the escape phase of cancer. By those means, pigs and dogs have the potential to contribute very differently to some of the unmet clinical needs within immuno-oncology.

Despite the growing interest in large animal models for biomedical research, a major limitation to distributing the use of both canine and porcine models for immuno-oncology lies within the reduction in funding provided for veterinary immunological research. Although the large animal models presented here offer promising in vivo systems for testing human anticancer therapies, they are labor-intensive, time-consuming, and expensive compared to rodents. Moreover, large animal models encompass additional challenges relating to housing, ethical regulation, and breeding difficulties as well as a limited number of commercially available reagents. For this reason, there is a need for specific calls addressing the continued development of immune relevant large animal cancer models, which will also secure a continued expansion of both the canine and porcine immunological toolboxes in addition to training of translational onco-immunologists. In conclusion, porcine and canine cancer models may complement unmet aspects of oncology research, but these large animal models should not replace the broad selection of mouse models, which continuously provide valuable knowledge to the research field. Instead, canine and porcine models offer a crucial link between mice and men; thus, choosing the appropriate combination of animal models for immuno-oncology research might increase the success rate when translating preclinical findings to the clinic.

Acknowledgments

Financial support. The authors thank the Danish Council for Independent Research, Technology and Production for the grant (ID: DFF-4005-00428) provided to GJ. Moreover, the authors acknowledge support from the Rural Development Administration, Republic of Korea (538JNU), the US NIH (1-R21-CA-195433-01A1) and the USAMRMC (CA150590) to LBS.

Potential conflicts of interest. All authors: No reported conflicts.

References

1. WHO | Estimates for 2000–2015. *WHO* 2017. Available at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html. Accessed July 11, 2017.
2. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res*. 1970;13:1–27.
3. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer*. 2006;6(1):24–37. doi:10.1038/nrc1782.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674. doi:10.1016/j.cell.2011.02.013.
5. Shankaran V, Ikeda H, Bruce AT, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature*. 2001;410(6832):1107–1111. doi:10.1038/35074122.
6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol*. 2002;3(11):991–998. doi:10.1038/ni1102-991.
7. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 2004;21(2):137–148. doi:10.1016/j.immuni.2004.07.017.
8. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol*. 2004;22(1):329–360. doi:10.1146/annurev.immunol.22.012703.104803.
9. Hadrup S, Donia M, Thor Straten P. Effector CD4 and CD8 T cells and their role in the tumor microenvironment. *Cancer Microenviron*. 2013;6(2):123–133. doi:10.1007/s12307-012-0127-6.
10. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*. 2011;331(6024):1565–1570. doi:10.1126/science.1203486.
11. van der Burg SH, Arens R, Ossendorp F, van Hall T, Melief CJM. Vaccines for established cancer: overcoming the challenges posed by immune evasion. *Nat Rev Cancer*. 2016;16(4):219–233. doi:10.1038/nrc.2016.16.

12. Kerbel RS. Tumor angiogenesis: past, present and the near future. *Carcinogenesis*. 2000;21(3):505–515. doi:10.1093/carcin/21.3.505.
13. Dighe AS, Richards E, Old LJ, Schreiber RD. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN gamma receptors. *Immunity*. 1994;1(6):447–456. doi:10.1016/1074-7613(94)90087-6.
14. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol*. 2006;90:1–50. doi:10.1016/S0065-2776(06)90001-7.
15. Loeb LA, Loeb KR, Anderson JP. Multiple mutations and cancer. *Proc Natl Acad Sci USA*. 2003;100(3):776–781. doi:10.1073/pnas.0334858100.
16. Takeda K, Nakayama M, Hayakawa Y, et al. IFN-γ is required for cytotoxic T cell-dependent cancer genome immunoediting. *Nat Commun*. 2017;8:14607. doi:10.1038/ncomms14607.
17. Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T. The urgent need to recover MHC class I in cancers for effective immunotherapy. *Curr Opin Immunol*. 2016;39:44–51. doi:10.1016/j.coi.2015.12.007.
18. Reiman JM, Kmieciak M, Manjili MH, Knutson KL. Tumor immunoediting and immunosculpting pathways to cancer progression. *Semin Cancer Biol*. 2007;17(4):275–287. doi:10.1016/j.semcancer.2007.06.009.
19. Kloor M, Becker C, Benner A, et al. Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. *Cancer Res*. 2005;65(14):6418–6424. doi:10.1158/0008-5472.CAN-05-0044.
20. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol*. 2007;25(1):267–296. doi:10.1146/annurev.immunol.25.022106.141609.
21. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature*. 1998;396(6712):643–649. doi:10.1038/25292.
22. Garrido F, Ruiz-Cabello F, Cabrera T, et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. *Immunol Today*. 1997;18(2):89–95. doi:10.1016/S0167-5699(96)10075-X.
23. Algarra I, Cabrera T, Garrido F. The HLA crossroad in tumor immunology. *Hum Immunol*. 2000;61(1):65–73. doi:10.1016/S0198-8859(99)00156-1.
24. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev*. 2001;182:18–32. doi:10.1034/j.1600-065×.2001.1820102.x.
25. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. *Nat Immunol*. 2002;3(11):999–1005. doi:10.1038/ni1102-999.
26. Kaplan DH, Shankaran V, Dighe AS, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. *Proc Natl Acad Sci USA*. 1998;95(13):7556–7561.
27. Zaidi MR, Merlino G. The two faces of interferon-γ in cancer. *Clin Cancer Res*. 2011;17(19):6118–6124. doi:10.1158/1078-0432.CCR-11-0482.
28. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol*. 2006;6(11):836–848. doi:10.1038/nri1961.
29. Pagès F, Galon J, Dieu-Nosjean M-C, Tartour E, Sautès-Fridman C, Fridman W-H. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene*. 2010;29(8):1093–1102. doi:10.1038/onc.2009.416.
30. Pagès F, Kirilovsky A, Mlecnik B, et al. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol*. 2009;27(35):5944–5951. doi:10.1200/JCO.2008.19.6147.
31. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298–306. doi:10.1038/nrc3245.
32. Griffin J. A strategic approach to vaccine development: animal models, monitoring vaccine efficacy, formulation and delivery. *Adv Drug Deliv Rev*. 2002;54(6):851–861. doi:10.1016/S0169-409×(02)00072-8.
33. Becher OJ, Holland EC. Genetically engineered models have advantages over xenografts for preclinical studies. *Cancer Res*. 2006;66(7):3355–3359. doi:10.1158/0008-5472.CAN-05-3827.
34. Zitvogel L, Pitt JM, Daillère R, Smyth MJ, Kroemer G. Mouse models in oncoimmunology. *Nat Rev Cancer*. 2016;16(12):759–773. doi:10.1038/nrc.2016.91.
35. Gillet J-P, Calcagno AM, Varma S, et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. *Proc Natl Acad Sci USA*. 2011;108(46):18708–18713. doi:10.1073/pnas.1111840108.
36. Schachtschneider KM, Schwind RM, Newson J, et al. The oncopig cancer model: an innovative large animal translational oncology platform. *Front Oncol*. 2017;7:190. doi:10.3389/FONC.2017.00190.
37. Suggitt M, Bibby MC. 50 years of preclinical anticancer drug screening: empirical to target-driven approaches. *Clin Cancer Res*. 2005;11(3):971–981.
38. Ostrand-Rosenberg S. Animal models of tumor immunity, immunotherapy and cancer vaccines. *Curr Opin Immunol*. 2004;16(2):143–150. doi:10.1016/j.coi.2004.01.003.
39. Sanmamed MF, Chester C, Melero I, Kohrt H. Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies. *Ann Oncol*. 2016;27(7):1190–1198. doi:10.1093/annonc/mdw041.
40. Borrell B. How accurate are cancer cell lines? *Nature*. 2010;463(7283):858–858. doi:10.1038/463858a.
41. Dranoff G. Experimental mouse tumour models: what can be learnt about human cancer immunology? *Nat Rev Immunol*. 2011;12(1):61–66. doi:10.1038/nri3129.
42. Sharpless NE, DePinho RA. The mighty mouse: genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov*. 2006;5(9):741–754. doi:10.1038/nrd2110.
43. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*. 2004;117(7):927–939. doi:10.1016/j.cell.2004.06.006.
44. Hoffman RM. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. *Invest New Drugs*. 1999;17(4):343–359.
45. Bibby MC. Orthotopic models of cancer for preclinical drug evaluation. *Eur J Cancer*. 2004;40(6):852–857. doi:10.1016/j.ejca.2003.11.021.
46. Zhao X, Li L, Starr TK, Subramanian S. Tumor location impacts immune response in mouse models of colon cancer. *Oncotarget*. 2017;8(33):54775–54787. doi:10.18632/oncotarget.18423.

47. Friedman D, Baird JR, Young KH, et al. Programmed cell death-1 blockade enhances response to stereotactic radiation in an orthotopic murine model of hepatocellular carcinoma. *Hepatol Res*. 2017;47(7):702–714. doi:10.1111/hepr.12789.
48. Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol*.2013;86(2):343–349. doi:10.1016/j.ijrobp.2012.12.025.
49. Oh T, Fakurnejad S, Sayegh ET, et al. Immunocompetent murine models for the study of glioblastoma immunotherapy. *J Transl Med*. 2014;12(1):107. doi:10.1186/1479-5876-12-107.
50. Connolly EM, Harmey JH, O'Grady T, et al. Cyclo-oxygenase inhibition reduces tumour growth and metastasis in an orthotopic model of breast cancer. *Br J Cancer*. 2002;87(2): 231–237. doi:10.1038/sj.bjc.6600462.
51. Oh K, Lee O-Y, Shon SY, et al. A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model. *Breast Cancer Res*. 2013; 15(5):R79. doi:10.1186/bcr3473.
52. Little EC, Wang C, Watson PM, Watson DK, Cole DJ, Camp ER. Novel immunocompetent murine models representing advanced local and metastatic pancreatic cancer. *J Surg Res*. 2012;176(2):359–366. doi:10.1016/j.jss.2011.10.025.
53. Soares KC, Foley K, Olino K, et al. A preclinical murine model of hepatic metastases. *J Vis Exp*. 2014;(91):51677. doi:10.3791/51677.
54. Tamura K, Kikuchi E, Konno T, et al. Therapeutic effect of intravesical administration of paclitaxel solubilized with poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) in an orthotopic bladder cancer model. *BMC Cancer*. 2015;15(1):317. doi:10.1186/s12885-015-1338-2.
55. Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. *Dis Model Mech*. 2008;1(2–3): 78–82. doi:10.1242/dmm.000976.
56. Merlino G, Flaherty K, Acquavella N, et al. Meeting report: the future of preclinical mouse models in melanoma treatment is now. *Pigment Cell Melanoma Res*. 2013;26(4):E8–E14. doi:10.1111/pcmr.12099.
57. Frese KK, Tuveson DA. Maximizing mouse cancer models. *Nat Rev Cancer*. 2007;7(9):654–658. doi:10.1038/nrc2192.
58. Gopinathan A, Tuveson DA. The use of GEM models for experimental cancer therapeutics. *Dis Model Mech*. 2008;1 (2–3):83–86. doi:10.1242/dmm.000570.
59. Walrath JC, Hawes JJ, Van Dyke T, Reilly KM. Genetically engineered mouse models in cancer research. *Adv Cancer Res*. 2010;106:113–164. doi:10.1016/S0065-230×(10)06004-5.
60. Lozano G, Behringer RR. New mouse models of cancer: single-cell knockouts. *Proc Natl Acad Sci USA*. 2007;104(11): 4245–4246. doi:10.1073/pnas.0700173104.
61. Wu X, Pandolfi PP. Mouse models for multistep tumorigenesis. *Trends Cell Biol*. 2001;11(11):S2–S9. doi:10.1016/S0962-8924(01)02127-4.
62. Day C-P, Merlino G, Van Dyke T. Preclinical mouse cancer models: a maze of opportunities and challenges. *Cell*. 2015; 163(1):39–53. doi:10.1016/j.cell.2015.08.068.
63. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. *Nat Protoc*. 2007;2(2): 247–250. doi:10.1038/nprot.2007.25.
64. Freeman A, Bridge JA, Maruthayanar P, et al. Comparative immune phenotypic analysis of cutaneous squamous cell carcinoma and intraepidermal carcinoma in immunecompetent individuals: proportional representation of CD8+ T-cells but not FoxP3+ regulatory T-cells is associated with disease stage. *PLoS One*. 2014;9(10):e110928. doi:10.1371/journal.pone.0110928.
65. Tentler JJ, Tan AC, Weekes CD, et al. Patient-derived tumour xenografts as models for oncology drug development. *Nat Rev Clin Oncol*. 2012;9(6):338–350. doi:10.1038/nrclinonc.2012.61.
66. Jung J. Human tumor xenograft models for preclinical assessment of anticancer drug development. *Toxicol Res*. 2014;30(1):1–5. doi:10.5487/TR.2014.30.1.001.
67. Kelland LR. Of mice and men: values and liabilities of the athymic nude mouse model in anticancer drug development. *Eur J Cancer*. 2004;40(6):827–836. doi:10.1016/j.ejca.2003.11.028.
68. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol*. 2007;7 (2):118–130. doi:10.1038/nri2017.
69. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science*. 2004;304(5667):104–107. doi:10.1126/science.1093933.
70. Ishikawa F, Yasukawa M, Lyons B, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor chainnull mice. *Blood*. 2005;106(5): 1565–1573. doi:10.1182/blood-2005-02-0516.
71. Shultz LD, Lyons BL, Burzenski LM, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol*. 2005;174(10):6477–6489. doi:10.4049/jimmunol.174.10.6477.
72. Carrillo MA, Zhen A, Kitchen SG. The use of the humanized mouse model in gene therapy and immunotherapy for HIV and cancer. *Front Immunol*. 2018;9:746. doi:10.3389/fimmu.2018.00746.
73. Suarez ER, Chang D-K, Sun J, et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. *Oncotarget*. 2016;7(23):34341–34355. doi:10.18632/oncotarget.9114.
74. Gay F, D'Agostino M, Giaccone L, et al. Immuno-oncologic approaches: CAR-T cells and checkpoint inhibitors. *Clin Lymphoma Myeloma Leuk*. 2017;17(8):471–478. doi:10.1016/j.clml.2017.06.014.
75. Cao X, Shores EW, Hu-Li J, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity*. 1995;2(3):223–238.
76. DiSanto JP, Müller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. *Proc Natl Acad Sci USA*. 1995;92(2):377–381.
77. Ohbo K, Suda T, Hashiyama M, et al. Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. *Blood*. 1996;87(3):956–967.
78. Bernard D, Peakman M, Hayday AC. Establishing humanized mice using stem cells: maximizing the potential. *Clin Exp Immunol*. 2008;152(3):406–414. doi:10.1111/j.1365-2249.2008.03659.x.
79. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA*. 2013;110(9):3507–3512. doi:10.1073/pnas.1222878110.
80. Mak IWY, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. *Am J Transl Res*. 2014;6(2):114–118. doi:AJTR1312010.

81. Wei W-Z, Jones RF, Juhasz C, Gibson H, Veenstra J. Evolution of animal models in cancer vaccine development. *Vaccine*. 2015;33(51):1–7. doi:10.1016/j.vaccine.2015.07.075.
82. Fleming JM, Creevy KE, Promislow DEL. Mortality in North American dogs from 1984 to 2004: an investigation into age-, size-, and breed-related causes of death. *J Vet Intern Med*. 2011;25(2):187–198. doi:10.1111/j.1939-1676.2011.0695.x.
83. Adams VJ, Evans KM, Sampson J, Wood JLN. Methods and mortality results of a health survey of purebred dogs in the UK. *J Small Anim Pract*. 2010;51(10):512–524. doi:10.1111/j.1748-5827.2010.00974.x.
84. Rowell JL, McCarthy DO, Alvarez CE. Dog models of naturally occurring cancer. *Trends Mol Med*. 2011;17(7):380–388. doi:10.1016/j.molmed.2011.02.004.
85. Pinho SS, Carvalho S, Cabral J, Reis CA, Gärtner F. Canine tumors: a spontaneous animal model of human carcinogenesis. *Transl Res*. 2012;159(3):165–172. doi:10.1016/j.trsl.2011.11.005.
86. Gardner HL, Fenger JM, London CA. Dogs as a model for cancer. *Annu Rev Anim Biosci*. 2016;4(1):199–222. doi:10.1146/annurev-animal-022114-110911.
87. Gordon I, Paoloni M, Mazcko C, Khanna C. The Comparative Oncology Trials Consortium: using spontaneously occurring cancers in dogs to inform the cancer drug development pathway. *PLoS Med*. 2009;6(10):e1000161. doi:10.1371/journal.pmed.1000161.
88. Król M, Motyl T. Exploiting cancer genomics in pet animals to gain advantage for personalized medicine decisions. *J Appl Genet*. 2014;55(3):337–341. doi:10.1007/s13353-014-0206-0.
89. Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Invest*. 2000;18(8):781–792.
90. Park JS, Withers SS, Modiano JF, et al. Canine cancer immunotherapy studies: linking mouse and human. *J Immunother cancer*. 2016;4(1):97. doi:10.1186/s40425-016-0200-7.
91. Lindblad-Toh K, Wade CM, Mikkelsen TS, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*. 2005;438(7069):803–819. doi:10.1038/nature04338.
92. Richards KL, Suter SE. Man's best friend: what can pet dogs teach us about non-Hodgkin's lymphoma? *Immunol Rev*. 2015;263(1):173–191. doi:10.1111/imr.12238.
93. Kirkness EF, Bafna V, Halpern AL, et al. The dog genome: survey sequencing and comparative analysis. *Science*. 2003;301(5641):1898–1903. doi:10.1126/science.1086432.
94. Cadieu E, Ostrander EA. Canine genetics offers new mechanisms for the study of human cancer. *Cancer Epidemiol Biomarkers Prev*. 2007;16(11):2181–2183. doi:10.1158/1055-9965.EPI-07-2667.
95. Angstadt AY, Thayanithy V, Subramanian S, Modiano JF, Breen M. A genome-wide approach to comparative oncology: high-resolution oligonucleotide aCGH of canine and human osteosarcoma pinpoints shared microaberrations. *Cancer Genet*. 2012;205(11):572–587. doi:10.1016/J.CANCERGEN.2012.09.005.
96. Angstadt AY, Motsinger-Reif A, Thomas R, et al. Characterization of canine osteosarcoma by array comparative genomic hybridization and RT-qPCR: signatures of genomic imbalance in canine osteosarcoma parallel the human counterpart. *Genes Chromosomes Cancer*. 2011;50(11):859–874. doi:10.1002/gcc.20908.
97. Paoloni M, Davis S, Lana S, et al. Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. *BMC Genomics*. 2009;10(1):625. doi:10.1186/1471-2164-10-625.
98. Breen M, Modiano JF. Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans-man and his best friend share more than companionship. *Chromosom Res*. 2008;16(1):145–154. doi:10.1007/s10577-007-1212-4.
99. Richards KL, Motsinger-Reif AA, Chen H-W, et al. Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. *Cancer Res*. 2013;73(16):5029–5039. doi:10.1158/0008-5472.CAN-12-3546.
100. Thomas R, Seiser EL, Motsinger-Reif A, et al. Refining tumor-associated aneuploidy through "genomic recoding" of recurrent DNA copy number aberrations in 150 canine non-Hodgkin lymphomas. *Leuk Lymphoma*. 2011;52(7):1321–1335. doi:10.3109/10428194.2011.559802.
101. Roode SC, Rotroff D, Avery AC, et al. Genome-wide assessment of recurrent genomic imbalances in canine leukemia identifies evolutionarily conserved regions for subtype differentiation. *Chromosome Res*. 2015;23(4):681–708. doi:10.1007/s10577-015-9475-7.
102. Shapiro SG, Raghunath S, Williams C, et al. Canine urothelial carcinoma: genomically aberrant and comparatively relevant. *Chromosome Res*. 2015;23(2):311–331. doi:10.1007/s10577-015-9471-y.
103. Hedan B, Thomas R, Motsinger-Reif A, et al. Molecular cytogenetic characterization of canine histiocytic sarcoma: a spontaneous model for human histiocytic cancer identifies deletion of tumor suppressor genes and highlights influence of genetic background on tumor behavior. *BMC Cancer*. 2011;11(1):201. doi:10.1186/1471-2407-11-201.
104. Thomas R, Duke SE, Wang HJ, et al. "Putting our heads together": insights into genomic conservation between human and canine intracranial tumors. *J Neurooncol*. 2009;94(3):333–349. doi:10.1007/s11060-009-9877-5.
105. Poorman K, Borst L, Moroff S, et al. Comparative cytogenetic characterization of primary canine melanocytic lesions using array CGH and fluorescence in situ hybridization. *Chromosome Res*. 2015;23(2):171–186. doi:10.1007/s10577-014-9444-6.
106. Uva P, Aurisicchio L, Watters J, et al. Comparative expression pathway analysis of human and canine mammary tumors. *BMC Genomics*. 2009;10(1):135. doi:10.1186/1471-2164-10-135.
107. Klopfleisch R, Lenze D, Hummel M, Gruber AD. Metastatic canine mammary carcinomas can be identified by a gene expression profile that partly overlaps with human breast cancer profiles. *BMC Cancer*. 2010;10(1):618. doi:10.1186/1471-2407-10-618.
108. Cobbold S, Metcalfe S. Monoclonal antibodies that define canine homologues of human CD antigens: summary of the First International Canine Leukocyte Antigen Workshop (CLAW). *Tissue Antigens*. 1994;43(3):137–154. doi:10.1111/j.1399-0039.1994.tb02315.x.
109. Isotani M, Katsuma K, Tamura K, et al. Efficient generation of canine bone marrow-derived dendritic cells. *J Vet Med Sci*. 2006;68(8):809–814. doi:10.1292/jvms.68.809.
110. Hartley AN, Tarleton RL. Chemokine receptor 7 (CCR7)-expression and IFNγ production define vaccine-specific canine T-cell subsets. *Vet Immunol Immunopathol*. 2015;164(3–4):127–136. doi:10.1016/j.vetimm.2015.02.001.
111. Ross P, Buntzman AS, Vincent BG, et al. Allelic diversity at the DLA-88 locus in Golden Retriever and Boxer breeds is limited. *Tissue Antigens*. 2012;80(2):175–183. doi:10.1111/j.1399-0039.2012.01889.x.

112. Angles JM, Kennedy LJ, Pedersen NC. Frequency and distribution of alleles of canine MHC-II DLA-DQB1, DLA-DQA1 and DLA-DRB1 in 25 representative American Kennel Club breeds. *Tissue Antigens*. 2005;66(3):173–184. doi:10.1111/j.1399-0039.2005.00461.x.
113. Ross P, Holmes JC, Gojanovich GS, Hess PR. A cell-based MHC stabilization assay for the detection of peptide binding to the canine classical class I molecule, DLA-88. *Vet Immunol Immunopathol*. 2012;150(3–4):206–212. doi:10.1016/j.vetimm.2012.08.012.
114. Estrela-Lima A, Araújo MS, Costa-Neto JM, et al. Immunophenotypic features of tumor infiltrating lymphocytes from mammary carcinomas in female dogs associated with prognostic factors and survival rates. *BMC Cancer*. 2010;10(1):256. doi:10.1186/1471-2407-10-256.
115. Raposo T, Gregório H, Pires I, Prada J, Queiroga FL. Prognostic value of tumour-associated macrophages in canine mammary tumours. *Vet Comp Oncol*. 2014;12(1):10–19. doi:10.1111/j.1476-5829.2012.00326.x.
116. Mitchell L, Dow SW, Slansky JE, Biller BJ. Induction of remission results in spontaneous enhancement of antitumor cytotoxic T-lymphocyte activity in dogs with B cell lymphoma. *Vet Immunol Immunopathol*. 2012;145(3–4):597–603. doi:10.1016/j.vetimm.2012.01.006.
117. Mucha J, Majchrzak K, Taciak B, Hellmén E, Król M. MDSCs mediate angiogenesis and predispose canine mammary tumor cells for metastasis via IL-28/IL-28RA (IFN-λ) signaling. *PLoS One*. 2014;9(7):e103249. doi:10.1371/journal.pone.0103249.
118. Tagawa M, Maekawa N, Konnai S, Takagi S. Evaluation of costimulatory molecules in peripheral blood lymphocytes of canine patients with histiocytic sarcoma. *PLoS One*. 2016;11(2):e0150030. doi:10.1371/journal.pone.0150030.
119. Maekawa N, Konnai S, Ikebuchi R, et al. Expression of PD-L1 on canine tumor cells and enhancement of IFN-γ production from tumor-infiltrating cells by PD-L1 blockade. *PLoS One*. 2014;9(6):e98415. doi:10.1371/journal.pone.0098415.
120. Maekawa N, Konnai S, Takagi S, et al. A canine chimeric monoclonal antibody targeting PD-L1 and its clinical efficacy in canine oral malignant melanoma or undifferentiated sarcoma. *Sci Rep*. 2017;7(1):8951. doi:10.1038/s41598-017-09444-2.
121. Maekawa N, Konnai S, Okagawa T, et al. Immunohistochemical analysis of PD-L1 expression in canine malignant cancers and PD-1 expression on lymphocytes in canine oral melanoma. *PLoS One*. 2016;11(6):e0157176. doi:10.1371/journal.pone.0157176.
122. Coy J, Caldwell A, Chow L, Guth A, Dow S. PD-1 expression by canine T cells and functional effects of PD-1 blockade. *Vet Comp Oncol*. 2017;15(4):1487–1502. doi:10.1111/vco.12294.
123. Hartley G, Faulhaber E, Caldwell A, et al. Immune regulation of canine tumour and macrophage PD-L1 expression. *Vet Comp Oncol*. 2017;15(2):534–549. doi:10.1111/vco.12197.
124. Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med*. 2011;3(95):95ra73. doi:10.1126/scitranslmed.3002842.
125. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725–733. doi:10.1056/NEJMoa1103849.
126. Mata M, Vera JF, Gerken C, et al. Toward immunotherapy with redirected T cells in a large animal model. *J Immunother*. 2014;37(8):407–415. doi:10.1097/CJI.0000000000000052.
127. Panjwani MK, Smith JB, Schutsky K, et al. Feasibility and safety of RNA-transfected CD20-specific chimeric antigen receptor T cells in dogs with spontaneous B cell lymphoma. *Mol Ther*. 2016;24(9):1602–1614. doi:10.1038/mt.2016.146.
128. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther*. 2010;18(4):843–851. doi:10.1038/mt.2010.24.
129. Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol*. 2004;4(1):46–55. doi:10.1038/nri1260.
130. Qu C, Chen T, Fan C, et al. Efficacy of neonatal HBV vaccination on liver cancer and other liver diseases over 30-year follow-up of the Qidong Hepatitis B Intervention Study: a cluster randomized controlled trial. *PLoS Med*. 2014;11(12):e1001774. doi:10.1371/journal.pmed.1001774.
131. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411–422. doi:10.1056/NEJMoa1001294.
132. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer*. 2009;115(16):3670–3679. doi:10.1002/cncr.24429.
133. U'Ren LW, Biller BJ, Elmslie RE, Thamm DH, Dow SW. Evaluation of a novel tumor vaccine in dogs with hemangiosarcoma. *J Vet Intern Med*. 2007;21(1):113–120. doi:10.1111/j.1939-1676.2007.tb02936.x.
134. Andersen BM, Pluhar GE, Seiler CE, et al. Vaccination for invasive canine meningioma induces in situ production of antibodies capable of antibody-dependent cell-mediated cytotoxicity. *Cancer Res*. 2013;73(10):2987–2997. doi:10.1158/0008-5472.CAN-12-3366.
135. Pluhar GE, Grogan PT, Seiler C, et al. Anti-tumor immune response correlates with neurological symptoms in a dog with spontaneous astrocytoma treated by gene and vaccine therapy. *Vaccine*. 2010;28(19):3371–3378. doi:10.1016/j.vaccine.2010.02.082.
136. Anderson K, Modiano J. Progress in adaptive immunotherapy for cancer in companion animals: success on the path to a cure. *Vet Sci*. 2015;2(4):363–387. doi:10.3390/vetsci2040363.
137. Atherton MJ, Morris JS, McDermott MR, Lichty BD. Cancer immunology and canine malignant melanoma: a comparative review. *Vet Immunol Immunopathol*. 2016;169:15–26. doi:10.1016/j.vetimm.2015.11.003.
138. Regan D, Guth A, Coy J, Dow S. Cancer immunotherapy in veterinary medicine: current options and new developments. *Vet J*. 2016;207:20–28. doi:10.1016/j.tvjl.2015.10.008.
139. Mason NJ, Gnanandarajah JS, Engiles JB, et al. Immunotherapy with a HER2-targeting listeria induces HER2-specific immunity and demonstrates potential therapeutic effects in a phase I trial in canine osteosarcoma. *Clin Cancer Res*. 2016;22(17):4380–4390. doi:10.1158/1078-0432.CCR-16-0088.
140. Mito K, Sugiura K, Ueda K, et al. IFN markedly cooperates with intratumoral dendritic cell vaccine in dog tumor models. *Cancer Res*. 2010;70(18):7093–7101. doi:10.1158/0008-5472.CAN-10-0600.
141. Dawson HD, Loveland JE, Pascal G, et al. Structural and functional annotation of the porcine immunome. *BMC Genomics*. 2013;14:332. doi:10.1186/1471-2164-14-332.
142. Groenen MAM, Archibald AL, Uenishi H, et al. Analyses of pig genomes provide insight into porcine demography and

evolution. *Nature*. 2012;491(7424):393–398. doi:10.1038/nature11622.
143. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human infectious diseases. *Trends Microbiol*. 2012;20(1):50–57. doi:10.1016/j.tim.2011.11.002.
144. Swindle MM, Makin A, Herron AJ, Clubb FJ, Frazier KS. Swine as models in biomedical research and toxicology testing. *Vet Pathol*. 2012;49(2):344–356. doi:10.1177/0300985811402846.
145. Helke KL, Nelson KN, Sargeant AM, et al. Pigs in toxicology. *Toxicol Pathol*. 2016;44(4):575–590. doi:10.1177/0192623316639389.
146. Helke KL, Swindle MM. Animal models of toxicology testing: the role of pigs. *Expert Opin Drug Metab Toxicol*. 2013;9(2):127–139. doi:10.1517/17425255.2013.739607.
147. Ho C-S, Lunney JK, Ando A, et al. Nomenclature for factors of the SLA system, update 2008. *Tissue Antigens*. 2009;73(4):307–315. doi:10.1111/j.1399-0039.2009.01213.x.
148. Flisikowska T, Kind A, Schnieke A. The new pig on the block: modelling cancer in pigs. *Transgenic Res*. 2013;22(4):673–680. doi:10.1007/s11248-013-9720-9.
149. Watson AL, Carlson DF, Largaespada DA, Hackett PB, Fahrenkrug SC. Engineered swine models of cancer. *Front Genet*. 2016;7:78. doi:10.3389/fgene.2016.00078.
150. Schachtschneider KM, Madsen O, Park C, Rund LA, Groenen MAM, Schook LB. Adult porcine genome-wide DNA methylation patterns support pigs as a biomedical model. *BMC Genomics*. 2015;16(1):743. doi:10.1186/s12864-015-1938-x.
151. Choi M, Lee J, Le MT, et al. Genome-wide analysis of DNA methylation in pigs using reduced representation bisulfite sequencing. *DNA Res*. 2015;22(5):343–355. doi:10.1093/dnares/dsv017.
152. Schook LB, Beever JE, Rogers J, et al. Swine Genome Sequencing Consortium (SGSC): a strategic roadmap for sequencing the pig genome. *Comp Funct Genomics*. 2005;6(4):251–255. doi:10.1002/cfg.479.
153. Paoloni MC, Khanna C. Comparative oncology today. *Vet Clin North Am Small Anim Pract*. 2007;37(6):1023–1032. doi:10.1016/j.cvsm.2007.08.003.
154. Breen M. Update on genomics in veterinary oncology. *Top Companion Anim Med*. 2009;24(3):113–121. doi:10.1053/j.tcam.2009.03.002.
155. Schook L, Beattie C, Beever J, et al. Swine in biomedical research: creating the building blocks of animal models. *Anim Biotechnol*. 2005;16(2):183–190. doi:10.1080/10495390500265034.
156. Gray MA, Pollock CB, Schook LB, Squires EJ. Characterization of porcine pregnane X receptor, farnesoid X receptor and their splice variants. *Exp Biol Med*. 2010;235(6):718–736. doi:10.1258/ebm.2010.009339.
157. Pollock CB, Rogatcheva MB, Schook LB. Comparative genomics of xenobiotic metabolism: a porcine-human PXR gene comparison. *Mamm Genome*. 2007;18(3):210–219. doi:10.1007/s00335-007-9007-7.
158. Gün G, Kues WA. Current progress of genetically engineered pig models for biomedical research. *Biores Open Access*. 2014;3(6):255–264. doi:10.1089/biores.2014.0039.
159. Pathak S, Multani AS, McConkey DJ, Imam AS, Amoss MS. Spontaneous regression of cutaneous melanoma in sinclair swine is associated with defective telomerase activity and extensive telomere erosion. *Int J Oncol*. 2000;17(6):1219–1224. doi:10.3892/ijo.17.6.1219.
160. Stewart SA, Weinberg RA. Telomerase and human tumorigenesis. *Semin Cancer Biol*. 2000;10(6):399–406. doi:10.1006/scbi.2000.0339.
161. Dawson HD, Smith AD, Chen C, Urban JF. An in-depth comparison of the porcine, murine and human inflammasomes; lessons from the porcine genome and transcriptome. *Vet Microbiol*. 2017;202:2–15. doi:10.1016/j.vetmic.2016.05.013.
162. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol*. 2016;17(9):1025–1036. doi:10.1038/ni.3518.
163. Glasner A, Ghadially H, Gur C, et al. Recognition and prevention of tumor metastasis by the NK receptor NKp46/NCR1. *J Immunol*. 2012;188(6):2509–2515. doi:10.4049/jimmunol.1102461.
164. Piriou-guzylack L, Salmon H. Review article membrane markers of the immune cells in swine: an update. *Vet Res*. 2008;39(54). doi:10.1051/vetres:2008030.
165. Charerntantanakul W, Roth JA. Biology of porcine T lymphocytes. *Anim Health Res Rev*. 2006;7(1–2):81–96. doi:10.1017/S1466252307001235.
166. Käser T, Gerner W, Hammer SE, Patzl M, Saalmüller A. Phenotypic and functional characterisation of porcine CD4+CD25high regulatory T cells. *Vet Immunol Immunopathol*. 2008;122(1–2):153–158. doi:10.1016/j.vetimm.2007.08.002.
167. Gerner W, Käser T, Saalmüller A. Porcine T lymphocytes and NK cells-an update. *Dev Comp Immunol*. 2009;33(3):310–320. doi:10.1016/j.dci.2008.06.003.
168. Esin S, Shigematsu M, Nagai S, Eklund A, Wigzell H, Grunewald J. Different percentages of peripheral blood gamma delta + T cells in healthy individuals from different areas of the world. *Scand J Immunol*. 1996;43(5):593–596.
169. Saalmüller A, Reddehase MJ, Bühring HJ, Jonjić S, Koszinowski UH. Simultaneous expression of CD4 and CD8 antigens by a substantial proportion of resting porcine T lymphocytes. *Eur J Immunol*. 1987;17(9):1297–1301. doi:10.1002/eji.1830170912.
170. Overgaard NH, Jung J-W, Steptoe RJ, Wells JW. CD4+/CD8+ double-positive T cells: more than just a developmental stage? *J Leukoc Biol*. 2015;97(1):31–38. doi:10.1189/jlb.1RU0814-382.
171. Zuckermann FA, Husmann RJ. Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. *Immunology*. 1996;87(3):500–512.
172. Binns RM, Pabst R. Lymphoid tissue structure and lymphocyte trafficking in the pig. *Vet Immunol Immunopathol*. 1994;43(1–3):79–87. doi:10.1016/0165-2427(94)90123-6.
173. Bode G, Clausing P, Gervais F, et al. The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol Methods*. 2010;62(3):196–220. doi:10.1016/j.vascn.2010.05.009.
174. Sørensen MR, Ilsøe M, Strube ML, et al. Sequence-based genotyping of expressed swine leukocyte antigen class I alleles by next-generation sequencing reveal novel swine leukocyte antigen class I haplotypes and alleles in Belgian, Danish, and Kenyan fattening pigs and Göttingen minipigs. *Front Immunol*. 2017;8:701. doi:10.3389/fimmu.2017.00701.
175. Gao C, Quan J, Jiang X, Li C, Lu X, Chen H. Swine leukocyte antigen diversity in Canadian specific pathogen-free Yorkshire and Landrace Pigs. *Front Immunol*. 2017;8:282. doi:10.3389/fimmu.2017.00282.
176. Flisikowska T, Kind A, Schnieke A. Pigs as models of human cancers. *Theriogenology*. 2016;86(1):433–437. doi:10.1016/j.theriogenology.2016.04.058.
177. Boisgard R, Vincent-Naulleau S, Leplat J-J, et al. A new animal model for the imaging of melanoma: correlation of FDG PET with clinical outcome, macroscopic aspect and

histological classification in Melanoblastoma-bearing Libechov Minipigs. *Eur J Nucl Med Mol Imaging*. 2003;30(6):826–834. doi:10.1007/s00259-003-1152-y.
178. Grossi AB, Hyttel P, Jensen HE, Leifsson PS. Porcine melanotic cutaneous lesions and lymph nodes. *Vet Pathol*. 2015;52(1):83–91. doi:10.1177/0300985814521637.
179. Egidy G, Julé S, Bossé P, et al. Transcription analysis in the MeLiM swine model identifies RACK1 as a potential marker of malignancy for human melanocytic proliferation. *Mol Cancer*. 2008;7:34. doi:10.1186/1476-4598-7-34.
180. Suzuki S, Iwamoto M, Saito Y, et al. Il2rg gene-targeted severe combined immunodeficiency pigs. *Cell Stem Cell*. 2012;10(6):753–758. doi:10.1016/j.stem.2012.04.021.
181. Watanabe M, Nakano K, Matsunari H, et al. Generation of interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA. *PLoS One*. 2013;8(10):e76478. doi:10.1371/journal.pone.0076478.
182. Huang J, Guo X, Fan N, et al. RAG1/2 knockout pigs with severe combined immunodeficiency. *J Immunol*. 2014;193(3):1496–1503. doi:10.4049/jimmunol.1400915.
183. Ito T, Sendai Y, Yamazaki S, et al. Generation of recombination activating gene-1-deficient neonatal piglets: a model of T and B cell deficient severe combined immune deficiency. *PLoS One*. 2014;9(12):e113833. doi:10.1371/journal.pone.0113833.
184. Powell EJ, Graham J, Ellinwood NM, et al. T cell lymphoma and leukemia in severe combined immunodeficiency pigs following bone marrow transplantation: a case report. *Front Immunol*. 2017;8:813. doi:10.3389/fimmu.2017.00813.
185. Lee K, Kwon D-N, Ezashi T, et al. Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated RAG2 and accompanying severe combined immunodeficiency. *Proc Natl Acad Sci USA*. 2014;111(20):7260–7265. doi:10.1073/pnas.1406376111.
186. McCalla-Martin AC, Chen X, Linder KE, Estrada JL, Piedrahita JA. Varying phenotypes in swine versus murine transgenic models constitutively expressing the same human Sonic hedgehog transcriptional activator, K5-HGLI2ΔN. *Transgenic Res*. 2010;19(5):869–887. doi:10.1007/s11248-010-9362-0.
187. Flisikowska T, Merkl C, Landmann M, et al. A porcine model of familial adenomatous polyposis. *Gastroenterology*. 2012;143(5):1173–1175.e7. doi:10.1053/j.gastro.2012.07.110.
188. Tan W, Carlson DF, Lancto CA, et al. Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proc Natl Acad Sci USA*. 2013;110(41):16526–16531. doi:10.1073/pnas.1310478110.
189. Yamakawa H, Nagai T, Harasawa R, et al. Production of transgenic pig carrying MMTV/v-Ha-ras. *J Reprod Dev*. 1999;45(2):111–118. doi:10.1262/jrd.45.111.
190. Luo Y, Li J, Liu Y, et al. High efficiency of BRCA1 knockout using rAAV-mediated gene targeting: developing a pig model for breast cancer. *Transgenic Res*. 2011;20(5):975–988. doi:10.1007/s11248-010-9472-8.
191. Schook LB, Collares TV, Hu W, et al. A genetic porcine model of cancer. *PLoS One*. 2015;10(7):e0128864. doi:10.1371/journal.pone.0128864.
192. Muller PAJ, Vousden KH. p53 mutations in cancer. *Nat Cell Biol*. 2013;15(1):2–8. doi:10.1038/ncb2641.
193. Sieren JC, Meyerholz DK, Wang X-J, et al. Development and translational imaging of a TP53 porcine tumorigenesis model. *J Clin Invest*. 2014;124(9):4052–4066. doi:10.1172/JCI75447.
194. Li S, Edlinger M, Saalfrank A, et al. Viable pigs with a conditionally-activated oncogenic KRAS mutation. *Transgenic Res*. 2015;24(3):509–517. doi:10.1007/s11248-015-9866-8.
195. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer*. 2011;11(11):761–774. doi:10.1038/nrc3106.
196. Rachagani S, Senapati S, Chakraborty S, et al. Activated $KrasG^{12}D$ is associated with invasion and metastasis of pancreatic cancer cells through inhibition of E-cadherin. *Br J Cancer*. 2011;104(6):1038–1048. doi:10.1038/bjc.2011.31.
197. Saalfrank A, Janssen K-P, Ravon M, et al. A porcine model of osteosarcoma. *Oncogenesis*. 2016;5(3):e210. doi:10.1038/oncsis.2016.19.
198. Schachtschneider KM, Schwind RM, Darfour-Oduro KA, et al. A validated, transitional and translational porcine model of hepatocellular carcinoma. *Oncotarget*. 2017;8(38):63620–63634. doi:10.18632/oncotarget.18872.
199. LeBlanc AK, Mazcko C, Brown DE, et al. Creation of an NCI comparative brain tumor consortium: informing the translation of new knowledge from canine to human brain tumor patients. *Neuro Oncol*. 2016;18(9):1209–1218. doi:10.1093/neuonc/now051.
200. MacEwen EG, Kurzman ID, Rosenthal RC, et al. Therapy for osteosarcoma in dogs with intravenous injection of liposome-encapsulated muramyl tripeptide. *J Natl Cancer Inst*. 1989;81(12):935–938. doi:10.1093/jnci/81.12.935.
201. Vail DM, Thamm DH, Reiser H, et al. Assessment of GS-9219 in a pet dog model of non-Hodgkin's lymphoma. *Clin Cancer Res*. 2009;15(10):3503–3510. doi:10.1158/1078-0432.CCR-08-3113.
202. London CA, Bernabe LF, Barnard S, et al. Preclinical evaluation of the novel, orally bioavailable Selective Inhibitor of Nuclear Export (SINE) KPT-335 in spontaneous canine cancer: results of a phase I study. *PLoS One*. 2014;9(2):e87585. doi:10.1371/journal.pone.0087585.
203. Joshi AD, Botham RC, Schlein LJ, et al. Synergistic and targeted therapy with a procaspase-3 activator and temozolomide extends survival in glioma rodent models and is feasible for the treatment of canine malignant glioma patients. *Oncotarget*. 2017;8(46):80124–80138. doi:10.18632/oncotarget.19085.
204. Botham RC, Roth HS, Book AP, Roady PJ, Fan TM, Hergenrother PJ. Small-molecule procaspase-3 activation sensitizes cancer to treatment with diverse chemotherapeutics. *ACS Cent Sci*. 2016;2(8):545–559. doi:10.1021/acscentsci.6b00165.
205. Peterson QP, Hsu DC, Novotny CJ, et al. Discovery and canine preclinical assessment of a nontoxic procaspase-3-activating compound. *Cancer Res*. 2010;70(18):7232–7241. doi:10.1158/0008-5472.CAN-10-0766.
206. Institute NC. RFA-CA-17-001: canine immunotherapy trials and correlative studies (U01). Available at: https://grants.nih.gov/grants/guide/rfa-files/RFA-CA-17-001.html. Accessed February 12, 2018.
207. Ito D, Frantz AM, Modiano JF. Canine lymphoma as a comparative model for human non-Hodgkin lymphoma: recent progress and applications. *Vet Immunol Immunopathol*. 2014;159(3–4):192–201. doi:10.1016/j.vetimm.2014.02.016.
208. Seelig D, Avery A, Ehrhart E, Linden M. The comparative diagnostic features of canine and human lymphoma. *Vet Sci*. 2016;3(4):11. doi:10.3390/vetsci3020011.
209. Fan T, Khanna C. Comparative aspects of osteosarcoma pathogenesis in humans and dogs. *Vet Sci*. 2015;2(4):210–230. doi:10.3390/vetsci2030210.

210. Simpson S, Dunning MD, de Brot S, Grau-Roma L, Mongan NP, Rutland CS. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics. *Acta Vet Scand*. 2017;59(1):71. doi:10.1186/s13028-017-0341-9.
211. Fenger JM, London CA, Kisseberth WC. Canine osteosarcoma: a naturally occurring disease to inform pediatric oncology. *ILAR J*. 2014;55(1):69–85. doi:10.1093/ilar/ilu009.
212. Morello E, Martano M, Buracco P. Biology, diagnosis and treatment of canine appendicular osteosarcoma: similarities and differences with human osteosarcoma. *Vet J*. 2011; 189(3):268–277. doi:10.1016/J.TVJL.2010.08.014.
213. Lutful Kabir FM, Alvarez CE, Bird RC. Canine mammary carcinomas: a comparative analysis of altered gene expression. *Vet Sci*. 2016;3(1):1. doi:10.3390/vetsci3010001.
214. Klopfleisch R, Klose P, Weise C, et al. Proteome of metastatic canine mammary carcinomas: similarities to and differences from human breast cancer †. *J Proteome Res*. 2010;9(12):6380–6391. doi:10.1021/pr100671c.
215. Herranz C, Fernández F, Martín-Ibáñez R, et al. Spontaneously arising canine glioma as a potential model for human glioma. *J Comp Pathol*. 2016;154(2–3):169–179. doi:10.1016/J.JCPA.2015.12.001.
216. Hicks J, Platt S, Kent M, Haley A. Canine brain tumours: a model for the human disease? *Vet Comp Oncol*. 2017;15(1): 252–272. doi:10.1111/vco.12152.
217. Bentley RT, Ahmed AU, Yanke AB, Cohen-Gadol AA, Dey M. Dogs are man's best friend: in sickness and in health. *Neuro Oncol*. 2016;19(3):312–322. doi:10.1093/neuonc/now109.
218. Nishiya A, Massoco C, Felizzola C, et al. Comparative aspects of canine melanoma. *Vet Sci*. 2016;3(4):7. doi:10.3390/vetsci3010007.
219. Gillard M, Cadieu E, De Brito C, et al. Naturally occurring melanomas in dogs as models for non-UV pathways of human melanomas. *Pigment Cell Melanoma Res*. 2014;27(1): 90–102. doi:10.1111/pcmr.12170.
220. Simpson RM, Bastian BC, Michael HT, et al. Sporadic naturally occurring melanoma in dogs as a preclinical model for human melanoma. *Pigment Cell Melanoma Res*. 2014;27(1): 37–47. doi:10.1111/pcmr.12185.
221. Dhawan D, Paoloni M, Shukradas S, et al. Comparative gene expression analyses identify luminal and basal subtypes of canine invasive urothelial carcinoma that mimic patterns in human invasive bladder cancer. *PLoS One*. 2015; 10(9):e0136688. doi:10.1371/journal.pone.0136688.
222. Knapp DW, Ramos-Vara JA, Moore GE, Dhawan D, Bonney PL, Young KE. Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. *ILAR J*. 2014;55(1):100–118. doi:10.1093/ilar/ilu018.
223. Schachtschneider KM, Liu Y, Mäkeläinen S, et al. Oncopig soft-tissue sarcomas recapitulate key transcriptional features of human sarcomas. *Sci Rep*. 2017;7(1):2624. doi:10.1038/s41598-017-02912-9.
224. Teng MWL, Galon J, Fridman W-H, Smyth MJ. From mice to humans: developments in cancer immunoediting. *J Clin Invest*. 2015;125(9):3338–3346. doi:10.1172/JCI80004.
225. High WA, Stewart D, Wilbers CRH, Cockerell CJ, Hoang MP, Fitzpatrick JE. Completely regressed primary cutaneous malignant melanoma with nodal and/or visceral metastases: a report of 5 cases and assessment of the literature and diagnostic criteria. *J Am Acad Dermatol*. 2005;53(1): 89–100. doi:10.1016/j.jaad.2005.03.006.
226. Papac RJ. Spontaneous regression of cancer: possible mechanisms. *In Vivo*. 1996;12(6):571–578. doi:10.1.1.326.4921.
227. Flisikowski K, Flisikowska T, Sikorska A, et al. Germline gene polymorphisms predisposing domestic mammals to carcinogenesis. *Vet Comp Oncol*. 2017;15(2):289–298. doi:10.1111/vco.12186.
228. Vincent-Naulleau S, Le Chalony C, Leplat J-J, et al. Clinical and histopathological characterization of cutaneous melanomas in the melanoblastoma-bearing Libechov minipig model. *Pigment Cell Res*. 2004;17(1):24–35. doi:10.1046/j.1600-0749.2003.00101.x.
229. Canter RJ, Grossenbacher SK, Foltz JA, et al. Radiotherapy enhances natural killer cell cytotoxicity and localization in pre-clinical canine sarcomas and first-in-dog clinical trial. *J Immunother cancer*. 2017;5(1):98. doi:10.1186/s40425-017-0305-7.
230. Monjazeb AM, Kent MS, Grossenbacher SK, et al. Blocking indolamine-2,3-dioxygenase rebound immune suppression boosts antitumor effects of radio-immunotherapy in murine models and spontaneous canine malignancies. *Clin Cancer Res*. 2016;22(17):4328–4340. doi:10.1158/1078-0432.CCR-15-3026.
231. Dow S, Elmslie R, Kurzman I, MacEwen G, Pericle F, Liggitt D. Phase I study of liposome-DNA complexes encoding the interleukin-2 gene in dogs with osteosarcoma lung metastases. *Hum Gene Ther*. 2005;16(8):937–946. doi:10.1089/hum.2005.16.937.
232. Khanna C, Anderson PM, Hasz DE, Katsanis E, Neville M, Klausner JS. Interleukin-2 liposome inhalation therapy is safe and effective for dogs with spontaneous pulmonary metastases. *Cancer*. 1997;79(7):1409–1421.
233. Konietschke U, Teske E, Jurina K, Stockhaus C. Palliative intralesional interleukin-2 treatment in dogs with urinary bladder and urethral carcinomas. *In Vivo*. 2012; 26(6): 931–935.
234. Den Otter W, Hack M, Jacobs JJL, Tan JFV, Rozendaal L, Van Moorselaar RJA. Treatment of transmissible venereal tumors in dogs with intratumoral interleukin-2 (IL-2). A pilot study. *Anticancer Res*. 2015;35(2):713–717.
235. O'Connor CM, Sheppard S, Hartline CA, et al. Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy. *Sci Rep*. 2012;2(1):249. doi:10.1038/srep00249.
236. Mie K, Shimada T, Akiyoshi H, Hayashi A, Ohashi F. Change in peripheral blood lymphocyte count in dogs following adoptive immunotherapy using lymphokine-activated T killer cells combined with palliative tumor resection. *Vet Immunol Immunopathol*. 2016;177:58–63. doi:10.1016/j.vetimm.2016.06.007.
237. Mata M, Vera JF, Gerken C, et al. Toward immunotherapy with redirected T cells in a large animal model: ex vivo activation, expansion, and genetic modification of canine T cells. *J Immunother*. 2014;37(8):407–415. doi:10.1097/CJI.0000000000000052.
238. Gavazza A, Lubas G, Fridman A, et al. Safety and efficacy of a genetic vaccine targeting telomerase plus chemotherapy for the therapy of canine B-cell lymphoma. *Hum Gene Ther*. 2013;24(8):728–738. doi:10.1089/hum.2013.112.
239. Peruzzi D, Gavazza A, Mesiti G, et al. A vaccine targeting telomerase enhances survival of dogs affected by B-cell lymphoma. *Mol Ther*. 2010;18(8):1559–1567. doi:10.1038/mt.2010.104.

240. Finocchiaro LME, Fondello C, Gil-Cardeza ML, et al. Cytokine-enhanced vaccine and interferon-β plus suicide gene therapy as surgery adjuvant treatments for spontaneous canine melanoma. *Hum Gene Ther*. 2015;26(6): 367–376. doi:10.1089/hum.2014.130.

241. Bergman PJ, McKnight J, Novosad A, et al. Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. *Clin Cancer Res*. 2003;9(4): 1284–1290.