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The promise of immunotherapeutic strategies to advance cancer treatment in pet dogs

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ABSTRACT

In this article, which is part of the Currents in One Health series, principles of immunotherapeutics are discussed and their clinical exploration in dogs reviewed with emphasis on their translatability for improving treatment of commonly diagnosed cancers. With increasing longevity and sustained quality of life in pet dogs through dietary, environmental awareness, and preventative medical practices, the geriatric pet population has continued to steadily grow and, consequently, so have age-related pathologies. Not surprisingly, cancer is the most common cause of mortality in elderly dogs, accounting for 1 in 4 deaths in dogs > 10 years of age. Importantly, some cancer types that arise spontaneously in pet dogs are similar to cancers afflicting people. The shared clinical and biological behaviors of certain cancers observed in pet dogs and people underscore the opportunity to leverage comparative oncology studies, which can accelerate the validation and clinical implementation of innovative therapies that can benefit pet dogs and ultimately guide these strategies toward clinical practice in people too. In the era of immunotherapy, the inclusion of pet dogs that develop cancers under an intact immune system affords a unique and high-value opportunity to study the evolving nature of cancers shaped by immunosurveillance pressures. Complementing these discovery efforts and through a comparative oncology approach, the exploration and clinical validation of novel immunotherapeutic strategies in pet dogs can be foundational for defining the safety and immune-activating potential of new anticancer immune approaches that hold promise to transform cancer treatment in both pets and people alike.

Keywords: comparative oncology, immunotherapy, canine cancer, antitumor immunity, translational discovery

While cancer can arise from different cell types, bodily tissues, and internal organs, the fundamental underpinning of cancer development is essentially a corrupted cellular genome, either through altered structure or expression of coding genetic sequences.[1] Cellular genetic material (ie, DNA) can be altered following exposure to mutagens of either endogenous or exogenous source, and the risk for cancer development can be reduced through purposeful avoidance of exogenous mutagens (eg, tobacco use, alcohol consumption, environmental contaminants) by adopting healthier lifestyles.[2] However, endogenous mutagens such as reactive oxygen species produced during cellular respiration (eg, oxidative phosphorylation) and host defense (eg, neutrophil respiratory burst) are impossible to eliminate. Consequently, cancer risk generally increases over time as cells within the body are continuously exposed to both endogenous and exogenous insults with gradual accumulation of DNA mutations that tip the scale toward cancer formation **(Figure 1)**.[3]

If simply living increases risk for cancer following endogenous and exogenous mutagen exposures, what cellular fail-safes are erected to protect against cancer development? Fortunately, multiple defenses thwart cancer development, including DNA repair systems that surveil the genome, minimizing cumulative genetic mutations that favor cancer development.[4] However, inevitably some DNA errors can go unchecked and result in mutated cells. Luckily, the immune system plays an important role in immunosurveillance and is capable of recognizing and eliminating premalignant or nascent cancer cells before they can establish a strong foothold in their immediate surroundings known as the tumor microenvironment (TME).[5]

The immune system is complex, composed of physical (eg, skin), mechanical (eg, mucociliary apparatus), soluble (eg, cytokines), and cellular (eg, lymphocytes) components **(Figure 2)**.[6] The immune system can be divided into innate and adaptive arms,

Received August 17, 2024
Accepted September 30, 2024
Published online October 11, 2024

doi.org/10.2460/javma.24.08.0532

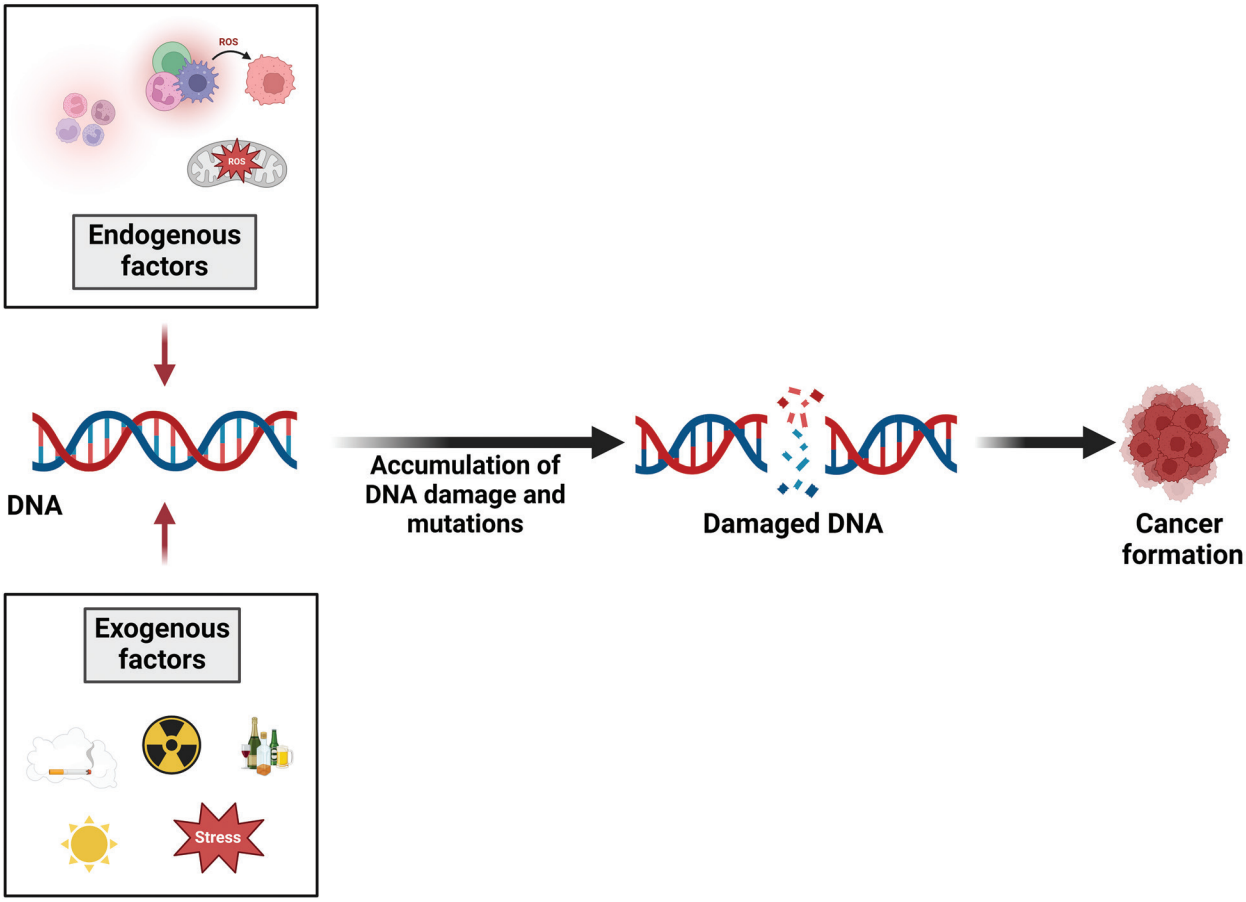

Figure 1—Endogenous and exogenous mutagens collaborate to damage DNA with longitudinal accumulation of mutations that favor the risk of cancer formation. Genomic stability is favored through the cooperative activities of DNA repair systems and active immunosurveillance mechanisms. Created with BioRender.com.

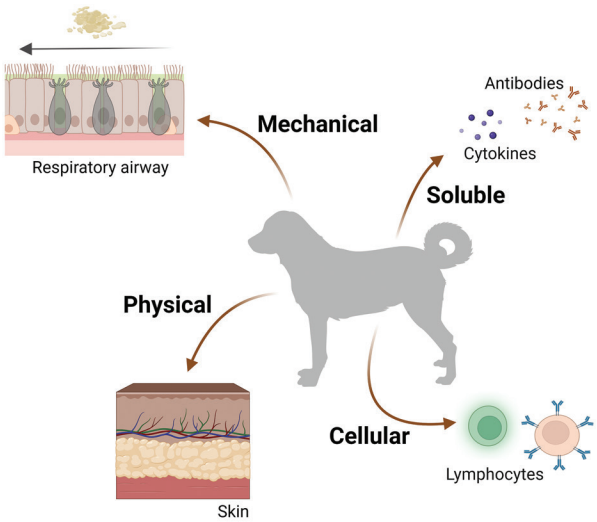

Figure 2—The immune system protects the host organism through multiple complementary mechanisms including physical barriers, mechanical transport movements, soluble factors, and cellular defenders. Collectively, all components of the immune system comprising the innate and adaptive arms work collaboratively and synergistically. Created with BioRender.com.

which feature complementary activities protecting host organisms from diverse pathogens. The main feature of innate immunity is to respond quickly and broadly (ie, nonspecifically) to pathogens through the generation of inflammation, with consequent priming of the adaptive immune response. The adaptive arm is more specialized and largely composed of lymphoid cells, which are capable of recognizing pathogens with specificity and memory. Importantly, the potency of adaptive immune responses is driven by the intensity of innate immune reactivity, and these 2 arms of immunity operate cooperatively and synergistically.[7]

While evolved to protect multicellular organisms from invading pathogens through the recognition of "nonself," the immune system can also recognize "altered self" (eg, tumor antigens) and can promote infiltration of immune cells into the TME with modulation of tumor progression.[8] Innate immune cells are composed of natural killer (NK) cells, neutrophils, and phagocytic cells (eg, macrophages) and suppress cancer development by either killing tumor cells directly or triggering the adaptive immune response.[9] The adaptive immune system relies on B and T lymphocytes to exert anticancer activities through the secretion of antibodies and cell-mediated

immune responses.[10] Unfortunately, even with operative innate and adaptive immune activities, cancer cells exploit mechanisms to evade immune recognition and destruction.[11] However, with an understanding of how the immune system can be amplified and reactivated, immunotherapy has the potential to revolutionize cancer management.

While breakthroughs in treating hematopoietic cancers (eg, lymphoma) have been successful using monoclonal antibodies (eg, anti-CD20) and genetic engineering (eg, chimeric antigen receptor T [CAR-T] cells), management of solid tumors (ie, sarcomas or carcinomas) has been less rewarding given roadblocks in generating immune responses strong enough to overcome the immunosuppressive shielding properties of the TME.[12] The TME provides a safe haven for nascent cancer cells through protective stromal (eg, collagens) and cellular (eg, regulatory T cells) elements. As such, immunotherapeutic interventions that manipulate the physical or cellular composition of the TME are likely key for improving treatment responses.[13] Classically, enhancing T-cell activation and effector functions for immunotherapy has been a major focus[14]; however, other immune cells (eg, macrophage) that are part of the innate and adaptive systems have been proven to contribute to immunotherapy responses, and there is tremendous opportunity to explore myeloid reprogramming strategies that favor M1 macrophage polarization to improve immunotherapeutic activities **(Figure 3)**.[15]

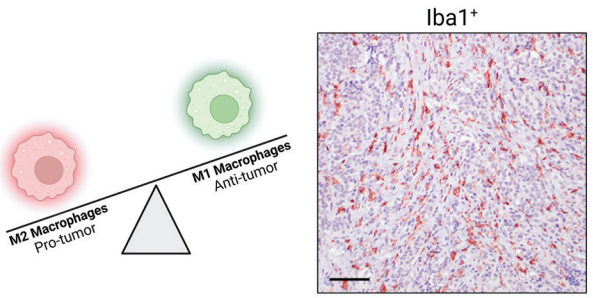

Figure 3—Solid tumors, such as canine oral malignant melanoma, are infiltrated with macrophages (Iba1+) typically with protumorigenic and immunosuppressive activities (M2 macrophages). Exploring myeloid reprogramming strategies to shift the balance toward antitumorigenic and proinflammatory activities (M1 macrophages) are hypothesized to synergize with T-cell–based therapies and improve immunotherapeutic outcomes. Created with BioRender.com.

Collectively, cancer immunotherapeutics can be categorized into 4 groups on the basis of mechanisms of activation[16] and include (1) passive and nonspecific (eg, NK cell therapy), (2) passive and specific (eg, monoclonal antibody, CAR-T cells), (3) active and nonspecific (eg, cytokines, checkpoint blockade), and (4) active and specific (eg, tumor vaccine). Some of these immunotherapeutic categories are further characterized subsequently; however, it is beyond the scope of this general review to detail all published studies to date and only a nonexhaustive list of specific strategies evaluated in pet dogs with cancer are described **(Figure 4)**.

Targeted Monoclonal Antibodies

Targeted monoclonal antibodies (MoAbs) bind to surface antigens expressed on cancer cells and can be unconjugated or conjugated with cytotoxic payloads (eg, antibody-drug conjugates) that promote tumor cell death through indirect and direct mechanisms.[17] Unconjugated MoAbs can induce tumor cell death by blocking critical growth factor receptor signaling, while indirect mechanisms can promote cell death via complement activation (ie, complement-dependent cytotoxicity), cellular killing (ie, antibody-dependent cellular cytotoxicity), or phagocytosis (ie, antibody-dependent cellular phagocytosis).[17,18] Conjugated MoAbs exert anticancer activities by bringing attached toxic payloads, including chemotherapeutics, radionuclides, or immunotoxins, in close proximity to kill cancer cells.[19]

In people, MoAbs have been developed to treat solid and hematopoietic tumors, with at least 43 FDA-approved antibody-based strategies to treat diverse tumor types. Importantly, the FDA approved rituximab (RITUXAN; Genentech) in 1997 as the first anti-CD20 monoclonal antibody for the treatment of diffuse large B-cell lymphoma, and this initial success story transformed the therapeutic landscape for incorporating MoAbs into the treatment of hematopoietic malignancies.[18] To date, first-line therapy for diffuse large B-cell lymphoma in people remains the combination of rituximab and multiagent chemotherapy, called R-CHOP, with the addition of rituximab increasing the 10-year event-free survival by 80% and overall survival by 60% compared to CHOP alone.[20]

Given the transformative impact of anti-CD20 MoAbs in human oncology, in conjunction with the high incidence of multicentric lymphoma in dogs,[21] tremendous interest exists for the clinical development of canine lymphoma-targeting MoAbs. To date, at least 2 noncommercialized canine-specific anti-CD20 MoAbs have been developed including 4E1-7-B and 1E4-cIgGB.[22–24] For 4E1-7-B, investigations have confirmed enhanced unconjugated cytotoxic properties including complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity in vitro against a canine B-cell lymphoma cell line (CLBL-1), as well as preclinical activity in a xenotransplant CLBL-1 mouse model. Furthermore, when 4E1-7-B was administered as a single IV injection (0.5 or 5.0 mg/kg) to Beagles, there was a dose-dependent, potent, and sustained depletion of circulating B lymphocytes for > 112 days, confirming on-target and immunobiologic activity of 4E1-7-B against peripheral circulating B cells. Recently, another antibody, 1E4-cIgGB, has been reported to be a biologic candidate for treating canine B-cell lymphoma. The 1E4-cIgGB antibody depletes B lymphocytes in Beagles[24] and has been shown to be well tolerated when administered in an adjuvant manner with doxorubicin and other immunomodulatory agents.[22] In the first-in-dog trial, 1E4-cIgGB was

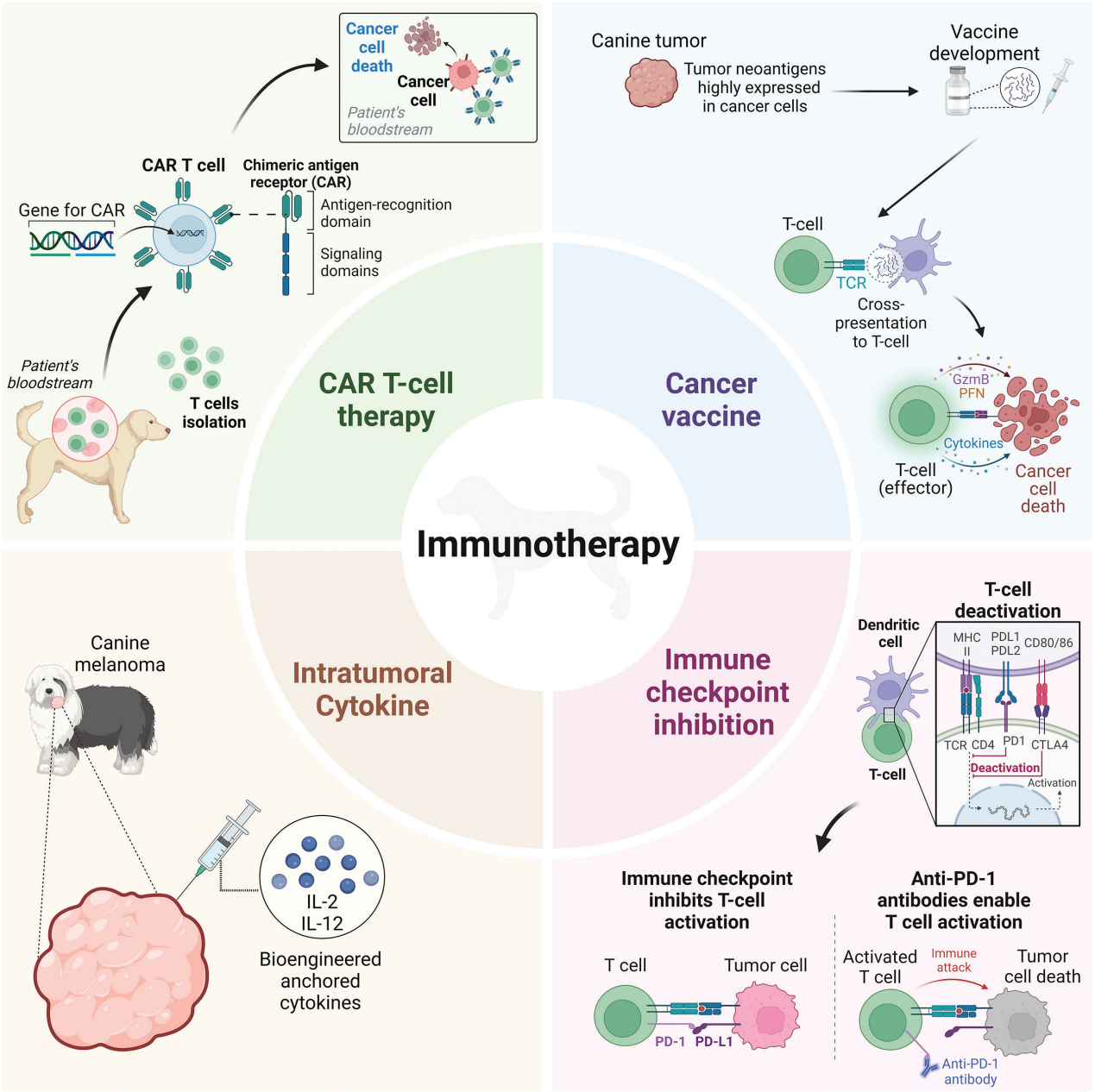

Figure 4—General classes of immunotherapeutic strategies that have strong scientific basis and have been explored clinically in the veterinary oncology patients include cancer vaccines, immune checkpoint inhibition with blocking monoclonal antibodies, chimeric antigen receptor (CAR)-T cells, and intratumoral cytokines. CTLA4 = Cytotoxic T-lymphocyte–associated protein 4. MHC II = Major histocompatibility complex class II. PD = Programmed cell death protein. PD-L = Programmed cell death ligand. TCR = T-cell receptor. Created with BioRender.com.

administered as a 90-minute IV infusion every 21 days for a total of 4 treatments, with the first initial loading dose at 20 mg/kg, followed with 3 subsequent doses at 10 mg/kg. Treatment with 1E4-cIgGB and doxorubicin potently reduced circulating B lymphocytes, and this effect was sustained with only 35% of dogs gradually reaching over 50% of their baseline circulating B lymphocyte count at day 196 of the trial.[22] While 1E4-cIgGB proved to be safe in combination with doxorubicin and other investigational agents and also depleted circulating B lymphocytes, prospective studies are required to evaluate its true antilymphoma activity when combined with traditional chemotherapy regimens such as CHOP.

Blocking Monoclonal Antibodies: Immune Checkpoint Inhibitors

Blocking MoAbs have revolutionized immunotherapy for solid tumors in people by "interrupting" counter-regulatory immune signaling that results in insufficient T-cell activation or effector T-cell exhaustion.[25]

Mechanistically, this class of MoAbs interferes with immune checkpoints, which are responsible for inhibitory signaling pathways that maintain immune tolerance. However, immune checkpoint signaling responsible for immune defervescence can be subverted by cancer cells to evade immune destruction.[26,27] Immune checkpoint inhibitors (ICIs), including MoAbs with blocking activities, amplify antitumor immune responses by interrupting coinhibitory signaling pathways with subsequent enhanced immune-mediated elimination of cancer cells.[26]

The most widely recognized targets for ICIs are cell surface molecules including cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death ligand 1 (PD-L1). Both CTLA-4 and PD-1 molecules are expressed on T cells and work to negatively regulate T-cell activation.[28,29] While performing similar functions, CTLA-4 is thought to regulate T-cell proliferation early in the immune response primarily in secondary lymphoid organs, while PD-1 suppresses T cells later in the immune response primarily in peripheral tissues including the TME. Given differing sites of activity, dual blockade of both CTLA-4 and PD-1 would likely exert synergistic effects leading to not only superior anticancer activities but also greater risk for immune-related toxicities.[30] Last, PD-L1, the cognate ligand for PD-1, is expressed by normal immune cells (eg, macrophages, dendritic cells, and activated lymphocytes) and epithelial cells and regulates immune tolerance by suppressing T-cell proliferation and cytokine secretion.[31] Tumor cells express PD-L1 as an adaptive immune mechanism to escape antitumor responses.[32] Inhibiting PD-1 or PD-L1 restores activity of T cells that have become quiescent and promotes the expansion of tumor-infiltrating lymphocytes that have an exhausted phenotype while reinvigorating their cytotoxic ability to induce tumor regression.[33,34] Underscoring the tremendous possibilities of checkpoint blockade strategies, in human oncology, there have been over 5,500 clinical trials evaluating the activity of ICI, and currently 7 checkpoint inhibitors are FDA approved for managing dozens of tumor types in people.

In veterinary oncology, the characterization of immune checkpoints has been reviewed recently,[35] including CTLA-4, PD-1, and PD-L1 expressions identified in over 15 different canine cancers. Despite several druggable targets, there has been particular interest in the PD-1/PD-L1 axis, with multiple investigations describing PD-L1 expressions in canine tumors including oral malignant melanoma, urothelial carcinoma, osteosarcoma, squamous cell carcinoma, nasal adenocarcinoma, and soft tissue sarcoma.[36–39] Building upon these descriptive studies, the safety and efficacy of checkpoint blockade using a chimeric anti–PD-L1 monoclonal antibody (c4G12) or a caninized anti-canine PD-1 antibody (ca-4F12-E6) have been explored, mostly for malignant melanoma.[36] As expected with potent immunotherapeutics, IV administration of c4G12 or ca-4F12-E6 could cause infusion reactions, and adverse events including transient fever and gastrointestinal upset were manifested by the majority (> 60%) of patients treated. Despite encountered immune adverse events, these seminal studies are promising, with objective response achieved in approximately 25% of patients evaluable,[36,40,41] which is comparable to that achieved in people with monotherapy regimens. Recently, a caninized monoclonal antibody Gilvetmab (Merck) that targets PD-1 on T cells[42] has been conditionally licensed by the USDA to treat dogs with stage I to III mast cell tumors and stage II to III malignant melanomas. In an unpublished field study, the overall response rate for dogs with stage I to III mast cell tumor or stage II to III malignant melanoma was 46% or 20%, respectively, and has led to Gilvetmab's commercialization and clinical use for treating pet dogs with cancer.

Cancer Vaccines

Cancer vaccines utilize tumor-specific antigens to generate T-cell–mediated antitumor immune responses and can be classified as prophylactic or therapeutic vaccines.[43,44] In people, 2 prophylactic cancer vaccines protecting against the hepatitis B virus that can cause hepatocellular carcinoma or human papillomavirus, which accounts for 70% of cervical cancer, have been approved by the FDA. Therapeutic cancer vaccines are used in patients who have developed cancer and are designed to strengthen patients' immune responses against existing cancer cells. At least 4 therapeutic cancer vaccines are available in people for the treatment of bladder cancer (Bacillus Calmette-Guérin and nadofaragene firadenovec [Adstiladrin; Ferring Pharmaceuticals]), prostate cancer (sipuleucel-T [Provenge; Dendreon Pharmaceuticals LLC]), and malignant melanoma (talimogene laherparepvec, also known as T-VEC or Imlygic; BioVex Inc).

In veterinary oncology, there has been tremendous interest in exploring the possibility of a pan-cancer preventative vaccine strategy. With advancements in genomic sequencing technologies, the identification of neoantigens derived from RNA processing errors conserved across multiple different tumor types in different species has become possible,[45] and it has been hypothesized that these shared neoantigens expressed exclusively by tumors are likely immunogenic and recognizable by endogenous T cells. Interestingly, immunization with a panel of conserved neoantigens has been shown to increase cell- and humoral-mediated immune responses in laboratory Beagles.[46] Building upon these preclinical studies, an interventional clinical trial with over 800 pet dogs called the Vaccination Against Canine Cancer Study intends to evaluate the safety and efficacy of this immunization strategy for preventing tumor development in pet dogs. While initial reports confirm the safety of the preventative cancer vaccine, results for reducing the incidence of cancer in dogs will require longer-term follow-up before the vaccine's effectiveness can be concluded.[46]

Several therapeutic vaccine strategies that target cancer-enriched antigens including enzymes (eg,

telomerase, tyrosinase) and surface proteins (eg, HER2/neu, CD20, GD3) have been evaluated in pet dogs,[47–49] and a nonexhaustive review of some of these approaches is described to serve as an example of the collective research conducted in veterinary oncology.

In 2007, a canine melanoma vaccine called Oncept (Merial) was conditionally approved by the USDA for improving survival times of dogs with locally controlled stage II or III oral malignant melanoma. Oncept is a xenogeneic DNA vaccine that encodes for human tyrosinase, a glycoprotein essential for melanin synthesis and highly conserved across mammalian species. Clinically, dogs receiving Oncept can develop anti-human tyrosinase-specific humoral and cellular responses with the capacity to cross-react against canine tyrosinase,[50,51] with the hypothesis that induced immune cross-reactivity to canine tyrosinase can be sufficient for controlling micrometastatic melanoma progression. The initial safety study for Oncept showed activity as an adjuvant for locally controlled stage II or III oral malignant melanoma on the basis of improved survival times compared to historic control groups.[52,53] However, other retrospective studies following licensure of Oncept have not consistently demonstrated improved survival outcomes with the vaccine.[54–57] The Oncept vaccine has also been used in an off-label manner for malignant melanomas affecting other anatomic sites.[58–60]

A recombinant listeria vaccine was developed to induce HER2/neu-specific immunity in solid tumors including osteosarcoma.[61] This strategy was first evaluated with the use of a formulation (ADXS31-164; Advaxis) intended to be translated for the treatment of pediatric osteosarcoma and generated promising results, showing that ADXS31-164 could induce HER2-specific immunity in 15 of 18 dogs with osteosarcoma and resulting in an increase in median disease-free interval (615 days) and median survival (956 days) when compared to a historical control group.[62] Disappointingly, a follow-up study utilizing a specific veterinary formulation called canine osteosarcoma vaccine, live listeria vector (Elanco), resulted in unacceptable outcomes from a human health exposure concern with a subpopulation of canine patients culturing positive for listeria and posing a potential zoonotic risk.[63,64] On the basis of these potential biological hazards, further studies with this particular listeria vector immunotherapeutic have been halted.

Elias Animal Health has developed an activated cellular therapy based on treatment with an autologous cancer cell vaccine, adoptive cellular therapy, and adjuvant IL-2 for newly diagnosed canine osteosarcoma. The prescribed treatment regimen is multilayered and combinatorial, requiring (1) treatment with autologous cancer vaccination derived from osteosarcoma tissues obtained at limb amputation, (2) leukapheresis to harvest immune cells (ie, T cells) for ex vivo activation and expansion, and (3) reinfusion with their activated T-cell product and subcutaneous IL-2 injections. In an initial pilot study[65] with 14 dogs, treatment was well tolerated, with low-grade and transient toxicities noted. The survival outcomes of dogs were impressive, with a median survival time of 415 days and 2-year survival rate of 36%.[65] Building upon this initial study, Elias Animal Health completed its pivotal combined safety and efficacy study (ECI-OSA-04) with 100 dogs, in which activated cellular therapy was compared against the standard of care with adjuvant carboplatin therapy. While dogs receiving activated cellular therapy did not benefit as much as dogs receiving adjuvant carboplatin, there was support for biologic activity and the USDA determined that results from the conditional study demonstrated a reasonable expectation of efficacy.

Adoptive Cell Therapy

Adoptive cell therapy utilizes immune cells, particularly T cells, which are isolated, expanded, and then reinfused back into patients with the goal of augmenting antitumor immunity and eliminating cancer cells.[66,67] While many forms of adoptive cell therapy have been explored, most strategies focus on expanding either tumor-infiltrating lymphocyte (TIL)–derived T cells or T cells genetically engineered to express tumor recognition and activation receptors. Tumor-infiltrating lymphocyte therapy consists of isolating lymphocytes trafficking into tumors, expanding their numbers in culture, and then reinfusing expanded lymphocytes back into a patient. The net effect of TIL therapy is to generate a greater number of activated T cells ex vivo with subsequent reinfusion to enhance the body's antitumor immune response.[68]

Another approach to enhance T-cell recognition and attack of cancer cells is through genetic manipulations of T cells.[69–71] These genetic modifications can either be made to the T-cell receptor to promote recognition of specific tumor peptides embedded within major histocompatibility complex of dendritic cells or to the creation of an artificial receptor commonly referred as CAR, which bypasses the necessity of cognate T-cell receptor and major histocompatibility complex binding altogether. The first experiments with CAR-T cells involved genetically modified T cells that express immunoglobulin–T-cell receptor chimeric molecules as the functional receptors.[72] These first-generation CAR-T cells were unable to persist in the body following adoptive transfer, so newer-generation CAR-T cells were developed to include concurrent expressions of costimulatory molecules, such as CD28, that enhanced CAR-T cells' persistence and activity in the body.[73,74] Next, it was then clinically demonstrated that CD19-specific CD28/CD3-zeta dual-signaling CAR-T cells could achieve molecular remissions in human adults with acute lymphoblastic leukemia.[75] These seminal findings in leukemic patients propelled CAR-T therapies for the treatment of other hematopoietic cancers, and currently there are 6 FDA-approved CAR-T therapies for treating people with lymphoma, leukemia, or multiple myeloma. Despite activity in hematopoietic cancers, CAR-T therapy for solid tumors has largely been unrewarding, likely due to the physical and immunosuppressive barriers erected by the TME

that impede CAR-T–cell trafficking and sustained effector activities.

In veterinary oncology, CAR-T–cell therapy has been investigated in both hematologic and solid tumor malignancies. The first clinical trial of CAR-T–cell therapy was conducted in a pet dog with relapsed diffuse large B-cell lymphoma[76] receiving anti-canine CD20 CAR-T cells generated via mRNA electroporation. The treatment was well tolerated, and the dog demonstrated a modest but transient reduction in CD20+ cells within target lymph nodes. Following this seminal first-in-dog report, a follow-up trial with anti-canine CD20 CAR-T cells produced via lentiviral transduction was conducted in 5 canine patients with diffuse large B-cell lymphoma.[77] In this second study, antigen-specific killing of CD20+ cells was demonstrated; however, the in vivo persistence of infused CAR-T cells was poor and 1 patient experienced disease relapse with a population of CD20– tumor cells following antigen escape. In both trials, the generation of canine CAR-T cells utilized the genetic insertion of a mouse antigen-binding domain that recognized canine CD20 and elicited the development of canine anti-mouse immunoglobulins against the CAR-T cells, potentially contributing to reduced persistence.

For solid tumors, preclinical investigations have shown the feasibility to engineer canine CAR-T cells to recognize specific tumor epitopes including HER2 in osteosarcoma,[78] IR-13Rα2 in glioma,[79] and B7-H3 in multiple tumor histologies including osteosarcoma, melanoma, and transmissible venereal tumor.[80] As these tumor-associated antigens are conserved between canine and human tumors, comparative oncology studies could be leveraged to optimize CAR-T–cell therapies for people. Given the current barriers facing CAR-T–cell therapies for treatment of solid tumors in people, the exploration of novel CAR-T–cell strategies to improve penetration into the TME and reversal of immunosuppressive barriers could be piloted in pet dogs and generate high-value biologic data for optimizing CAR-T–cell strategies, as well as other innovative therapeutic approaches, which could benefit both pets and people alike **(Figure 5)**.

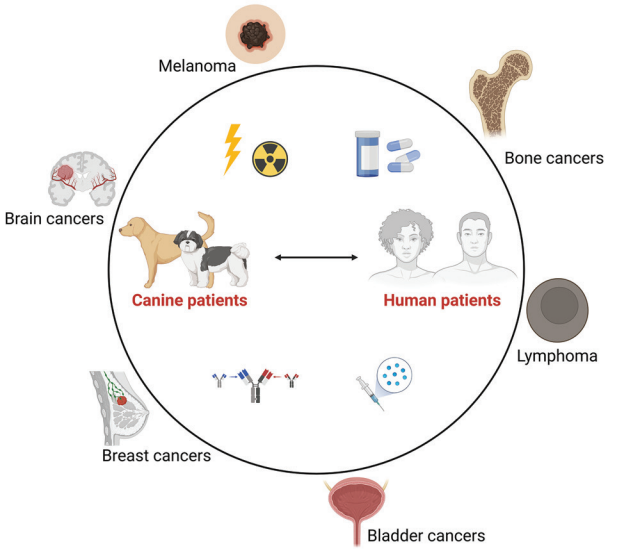

Figure 5—Comparative oncology approach allows for bidirectional discoveries that can benefit pet dogs and people with cancer. Inclusion of pet dogs in the evaluation of innovative therapies across 6 major tumor types of high comparative oncology relevance can accelerate translational impact for both pet dogs and people alike. Created with BioRender.com.

Cytokine Therapies

Cytokines are a diverse family of proteins including chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors that orchestrate cellular interactions throughout the immune system. Cytokines can be secreted by immune and nonimmune cells in response to cellular stresses such as infection, inflammation, and tumorigenesis, playing integral roles in amplifying innate and adaptive immune responses. While hundreds of cytokines have been identified to date, only 2 have been approved for the treatment of cancer in people and include interferon-α-2b (IFN-α) and IL-2.

Interferon-α is a type 1 interferon that exerts antiviral activities. The anticancer activities of IFN-α results from inducing senescence and apoptosis of cancer cells, in addition to promoting antitumor immune responses through enhanced dendritic cell maturation and T-cell cytotoxicity.[81] In people, IFN-α has therapeutic activity when administered at high doses for the treatment of chronic myeloid leukemia and melanoma.[82] Interleukin-2 is the prototypic anticancer cytokine that can expand T cells in vitro and in vivo, with subsequent promotion of anticancer immune-stimulatory properties.[83] As a cancer treatment, IL-2 is FDA approved for treating metastatic melanoma and renal cell carcinoma in humans due to its ability to increase NK cells and TILs.[84]

Despite exceptional anticancer activities in mouse tumor models, the broader application of cytokines in human cancer patients has been hindered by on-target, off-tumor, systemic proinflammatory effects resulting in vascular leak syndrome and other immune-related toxicities. Such has been the dilemma for translating IL-12 to the clinics,[85] despite profound tumor cytoreductive effects achieved in mouse tumor models. To reinvigorate the promise of cytokines as anticancer agents, protein engineering and material science techniques (eg, nanoparticles) are being actively explored to improve the local retention of cytokines within the TME, thereby reaping the anticancer benefits while minimizing systemic toxicities.

In veterinary medicine, type 1 interferons have been used for treatment of various allergic or infectious pathologies in dogs,[86] yet few studies have systematically evaluated their potential as anticancer agents.[87] However, the clinical assessment of IL-2 in dogs with cancer has been described since the mid-1990s and includes dogs with metastatic osteosarcoma, mast cell tumor, transmissible venereal tumors, and malignant melanoma.[88–90] Furthermore, different delivery methods for IL-2 such as intratumoral

injection, electrogene transfection, and aerosol liposome inhalation have been investigated.[88,89,91] Across these studies, there has been documented objective responses (as high as 50% in metastatic osteosarcoma) in treated dogs, underscoring the prospects of IL-2 as a cytokine for improving cancer management similar to what has been observed in people.

More recently, strategies to retain proinflammatory cytokines within the TME through protein engineering have gained wider attention as a means to mitigate systemic toxicities. Interestingly, some technological advancements in cytokine delivery have been evaluated in tumor-bearing dogs, with particular interest in evaluating the safety and activity of engineered IL-12, given its robust anticancer activities but life-limiting toxicity profile when administered systemically. In 1 study,[92] a novel construct called NHS-IL12, composed of IL-12 fused to an antibody that binds to exposed DNA released from necrotic cells, was given SC in dogs with malignant melanoma. NHS-IL12 exhibited dose-dependent immune-activating effects as measured by IFN-γ induction and also produced expected immune-related toxicities including reduced platelet counts, elevated liver enzymes, and vasculitis. Out of 18 dogs treated, 2 dogs achieved partial responses indicating some anticancer activities of NHS-IL12. Direct intratumoral injection of engineered IL-12 capable of being anchored within the TME or directly to tumor cells has emerged as promising strategies in pet dogs with cancer including malignant melanoma and soft tissue sarcoma.[93,94] Promisingly, through the delivery of intratumoral IL-12 and retention within the TME through GD2 binding, stromal collagen binding, or aluminum hydroxide binding, robust immune activation occurs within treated tumors and clinical objective responses are achieved in the absence of severe on-target off-tumor immune-related toxicities.[93–97] Excitingly, some of these technologies (eg, aluminum hydroxide–tethered IL-12 known as JEN-101; Jenga Biosciences) are being advanced toward veterinary commercialization to expand the swath of pet dogs that could benefit from these drug delivery innovations.

Conclusion

Cancer immunology is a rapidly evolving field, and despite steady advances in immunotherapies, there remain several challenges to overcome when aiming to effectively treat different forms of cancer. Some of these challenges include modest response rates, unpredictable clinical efficacy, and potential side effects such as autoimmune reactions or cytokine release syndromes. Tumor-infiltrating immune cells, particularly T cells, currently serve as the cellular underpinnings of most cancer immunotherapies. However, for many solid tumors, the largest fraction of immune infiltrating cells are macrophages, and a better understanding of myeloid cell programming and polarization in the TME is an exciting field of discovery that will likely yield additional immunotherapeutic targets for manipulation. Understanding the complexity of cancer and immune cells through multitiered modeling inclusive of pet dogs with naturally occurring cancers will provide further insights into cancer progression and propel the development and optimization of innovative immunotherapeutic strategies that can mutually benefit both pet dogs and people in the fight against cancer.

Acknowledgments

None reported.

Disclosures

Timothy M. Fan serves on the Scientific Advisory Board for Jenga Biosciences, a start-up veterinary company developing novel immunotherapeutics for veterinary oncologic applications.

No AI-assisted technologies were used in the generation of this manuscript.

Funding

The authors have nothing to disclose.

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