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CANINE MAMMARY CARCINOMA: CURRENT THERAPEUTIC TARGETS AND FUTURE PERSPECTIVES – A REVIEW

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Abstract

Canine mammary carcinoma (CMC) is the most common neoplasm in bitches, and it shares many biological similarities with breast cancer in humans. Drug resistance, high epigenetic mutations, and relapse rates are among the challenges which eventually urge the need for a veterinary oncologist to discover new therapeutic approaches that are more effective and safer. Therefore, in this review, we also cover the current therapeutic strategies from human medicine for the future perspectives of tumor immunotherapy in veterinary medicine. These strategies have great potential to be employed as therapeutic or prophylactic options due to their ability to modulate a specific and potent immune response against CMC. As we acquire a better understanding of canine tumor immunology, we can move towards a brighter prognosis. Additionally, we report on the recent successful studies in breast cancer that may benefit canines as well.

Key words: canine, mammary gland tumor, immunotherapy, neoplasm

Canine mammary carcinoma (CMC) is the most common cancer among intact bitches (*Canis familiaris*), worldwide (Goldschmidt et al., 2011). CMC has been linked to the development of a more malignant form later, which is more fatal (Sorenmo et al., 2000; Kristiansen et al., 2013). CMC can also occur in males; however, it is extremely rare, where it is 66 times less likely to happen and the reports of such occurrence are lacking (Saba et al., 2007). According to Vascellari et al. (2016), 56% of neoplasm cases among dogs were cases of CMC, whereby in every 100 000 dogs, 250 were diagnosed with CMC. The incidence is higher in certain breeds, such as English Spaniels, English Setter Spaniels, Poodle, and German Shepherds, while it is reportedly lower in Boxer breeds and Chihuahua (Baba and Catoi, 2007). The incidence of CMC is also correlated with age and hormonal imbalance (estradiol-17β, progesterone, and prolactin) (Spoerri et al., 2015). Intact dogs aged >9 years old showed the highest tendency to develop CMC (Sontas et al., 2009). However, some breeds, for example, the Springer Spaniel has been reported may develop much earlier with a mean age of 6.9 years old (Egenvall et al., 2005). The role of estrogen in influencing mammary carcinogenesis is largely linked (Canadas-Sousa et al., 2019). Exposure to ovarian hormones during the first two years of life is a well-known risk factor for breast cancer development (Schneider et al., 1969; Misdorp, 1988; Kristiansen et al., 2016). Estrogen and progesterone are necessary for regulating the sexual and reproductive functions of female organs including the uterus, mammary gland, and ovaries. The risk of tumor appearance is 0.5% before the first cycle, 8% after the first cycle, and more than 26% after the second or more estrous cycles (Baba and Catoi, 2007). It has been postulated that an increase in the estrogen level or overexposure to estrogen for a long time will increase the binding of the estrogen-to-estrogen receptor alpha (ER-α) ligands, thus activating gene transcription and genomic alteration (Canadas-Sousa et al., 2009; Mufudza et al., 2012). It is believed that estrogens can also activate hormones like relaxin to further induce cell

proliferation (Mufudza et al., 2012). While it is accepted that estrogen is dominantly involved in the development of CMC over progesterone (Mann et al., 2011; Fossum, 2013), it has been recorded that megestrol progestins may stimulate mammary neoplasia in beagle bitches where acetate progestin may stimulate mammary tumor development (Rutteman, 1992). Therefore, ovariohysterectomy is preferred over acetate progestin in controlling the population.

To date, more than 50% of the CMC cases among bitches are malignant (Sorenmo et al., 2000; Moe, 2001; Dias et al., 2016). CMC is cancer that majorly originates from the epithelial cells of the mammary gland, but mesenchymal and myoepithelial or mixed may present (Ramalho et al., 2006; Peña et al., 2014; Salas et al., 2015). Klopfleisch et al. (2010) reported that CMC shared similar gene expression with breast cancer in humans. It appears that DNA damage and DNA mutation are predominantly linked with the initiation step in the carcinogenesis of CMC, although environmental factors and host susceptibility may be involved as well. The comparison between canine and human mammary carcinoma is shown in Table 1.

The increasing incidence of cancer in animals merits continuous growth among veterinarians. In this review, we scrutinize the existing knowledge of canine therapeutics since these fields have not been reviewed in detail to the best of our knowledge. With the increasingly huge population of dogs globally, the prevalence of CMC also increases parallelly, thus diagnostic and therapeutic information is essential to address this problem as it is also related to animal welfare. Cancer rates in dogs are similar to those in humans, while the treatment and prophylactic options are limited in dogs (Weir et al., 2018). Additionally, CMC has been successfully exploited as a spontaneous model for breast cancer research in recent decades, and significant progress in veterinary oncology has been witnessed in terms of therapy and knowledge of this illness (Nguyen et al., 2018; Levi et al., 2021). Canine tends to develop cancer from middle age, with the majority of the canine species developing cancer at >9 years of age, which is equivalent to humans aged between 60–95, and this is when most malignancies are present in humans (Sultan and Ganaie, 2018; Schneider et al., 2018).

Table 1. Comparison between canine mammary tumor and human breast cancer

Parameters	Dogs	Human	References
Occurrence	Spontaneous tumor	Spontaneous tumor	(Abdelmegeed and Mohammed, 2018; Sultan and Ganaie, 2018)
Age susceptibility	Over 10 years old	Over 60 years old	(Fossum, 2013; Salas et al., 2015)
Etiology	Hormonal imbalance	Genetic mutation, unhealthy diet, and lifestyle	(Rivera et al., 2009; Polyak, 2007; Shah et al., 2014; Siegel et al., 2018; Gaddam et al., 2021)
Diagnosis	Physical examination, histology, and cytological examination of fine-needle aspirates (FNA) often become a golden standard	Imaging techniques (mammography, ultrasonography, magnetic resonance imaging, computed tomography) and biochemical biomarkers (nucleic acid hybridization system, real-time fluorescence quantitative PCR system, protein hybridization system, flow cytometer, needle biopsy, and immunohistochemistry)	(Simon et al., 2009; Sleekkx et al., 2011; He et al., 2020)
Germline mutation	Inherited mutations in the BRCA1 or BRCA2	Inherited mutations in the BRCA1 or BRCA2 *CHEK2*, *ATM* , *p53*, *PTEN*, *PALB2*, *RAD51C* and *RAD51D*	(Easton et al., 2007; Karami and Mehdipour, 2013; Sánchez-Bermudez et al., 2018; Abubakar, 2019; Brønden et al., 2003; Borge et al., 2011)
Predisposing risk	Unspayed bitch breed and genetic predisposition	Unhealthy lifestyle, genetics, stress, alcohol consumption, radiation, and infection	(Brønden et al., 2003; Borge et al., 2011)
Molecular markers	HER-2/neu, progesterone receptor (PR) positive/negative and estrogen receptor (ER) positive/negative, p53, Ki-67, epidermal growth factor receptor (EGFR)	HER-2/neu, progesterone receptor (PR) positive/negative and estrogen receptor (ER) positive/negative, p53, Ki-67, triple-negative breast cancer (ER−, PR− and HER-2/neu−), MUC1, EphA, Survivin, CEA, Wilms' tumor 1 (WT1)	(Queiroga et al., 2011; Rodel et al., 2012; Turriziani et al., 2012; Guo et al., 2015)

Diagnostic tests for canine mammary carcinoma

History, clinical signs, and physical examinations are the fundamentals in diagnosing a disease including CMC (Sleeckx et al., 2011). These are routine procedures for the veterinary clinician to palpate and examine the dogs from head to toe. In most cases, the mass is present superficially in the mammary region. A small nodule was commonly found in the inguinal mammary gland (Cheung et al., 2006; Jaillardon et al., 2012). Veterinary oncologists and general veterinary practitioners commonly use fine-needle biopsies of lymph nodes as a diagnostic tool (Sapierzyński et al., 2017). Fine needle aspiration (FNA) is to be performed as it is essential to glean material for cytological evaluation. FNA cytology is a rapid, simple, convenient, and inexpensive diagnostic procedure for CMC. Even though histology is sensitive and more accurate, however, cytology may be useful to provide a tentative diagnosis as it is a rapid and simple test, thus surgical intervention can be started to remove the mass as early as possible. In dogs, enlarged lymph nodes are regularly cytologically studied, and metastatic lymphadenomegaly of diverse origins is a common cytological result (Baker and Lumsden, 2000). McCourt et al. (2018) reported the distribution of the lymph node metastases from the CMC case.

Demonstration of metastasis of the sentinel lymph node (SLN), which is the first lymph node (or nodes) in the regional lymphatic basin to acquire lymphatic flow from the primary tumor, is crucial in the staging process and has a significant prognostic value (Yuen et al., 2004; Soultani et al., 2017). Using imaging modalities such as computed tomography (CT), ultrasonography, and magnetic resonance imaging (MRI), the size and shape of the SLN can provide criteria or indicators for metastatic invasion happening in the body (Yuen et al., 2004; Nakagawa et al., 2016). The majority of metastatic nodes displayed a heterogeneous pattern of spotted or partial or peripheral contrast opacification upon imaging (Soultani et al., 2017). Studies from Collivignarelli et al. (2021) reported that they successfully performed a good mapping for surgical extension and also accurate post-surgical prognosis based on SLN identification that benefited veterinary surgeons and oncologists. This SLN serves a crucial function as a filter and barrier for spreading tumor cells. However, the study also suggested that each dog with CMC should be examined case by case prior to surgery as lymph drainage patterns and SLN for each dog might differ.

Other distant nodes, such as the prescapular, popliteal, as well as sternal, and deep inguinal lymph nodes, were examined during the clinical examination of companion animals with CMC (Lana et al., 2007). FNA should be used to monitor any changes in size or consistency for cytological evaluation. In some CMC cases, there is an enlargement of the prescapular lymph node and there is a presence of a notable mass in the mammary region (Madewell et al., 1999). Madewell et al. (1999) reported a 13-year-old mixed breed bitch was presented to the veterinarian with a 2-week history of an enlarged right prescapular lymph node. In the study performed by Ku et al. (2017), metastatic neoplasms were found in as many as 40% of dogs and cats whose lymph nodes were analyzed. Lymphoma node metastases vary depending on the type of initial tumor; for example, they were found in 9–65% of malignant mammary carcinoma. In bitches with malignant mammary carcinoma, the occurrence of regional lymph node metastases was a significant prognostic marker (Szczubiał and Łopuszyński, 2011).

Histopathology and immunohistochemistry assessments are involved in classifying the grade of malignancies and providing a definitive diagnosis (Sorenmo et al., 2011; Rasotto et al., 2017). Since several studies have validated the prognostic significance of histopathological classification and grading, the new classification of CMC (Zappulli et al., 2019), which replaces the WHO's 1974 and 2011 classifications (Peña et al., 2013), and the current grading system used worldwide for malignant CMC as an adaptation of the Nottingham method used for HBC (Elston et al., 1991), have given pathologists tools for accurate diagnosis and prognosis of the patient. Perhaps, in terms of clinical practicality, the techniques are less attractive compared to FNA because they induce trauma due to the biopsy taken from the mass and consume a long time to obtain the result, however, in terms of specificity, histopathology and immunohistochemistry are more accurate and a gold standard in diagnosing CMC (Sorenmo et al., 2011; Rasotto et al., 2017). Moreover, every tumor will express specific proteins which are called tumor-associated antigens/tumor-specific antigens (TAAs/TSAs) (Mobasheri et al., 2010). These antigens are called biomarkers in tumor patients if they can be detected in other tissues, serum, or urine in quantities that differ from normal. Histopathology can reflect the existence of a remarkable resemblance in the origin, distribution, and behavior of neoplastic cells while immunohistochemistry can detect specific cancerous biomarkers (for example HER-2, P53, and Ki-67 markers) that are responsible for a specific type of mammary cancer (Al-Mansour et al., 2018). Moreover, it requires time for the results to be ready and they do not affect the decision on surgical treatment. Ventrodorsal and dorsoventral radiograph views are recommended to assess the metastasis level and determine whether cancer can metastasize to other organs.

Tumor microenvironment (TME) during CMC progression

The tumor microenvironment (TME) in solid tumors is a sophisticated and complex system that is composed of an acidic pH environment, endogenous hydrogen peroxide (H_2O_2), hypoxia, and the fluctuation in the expression of the extracellular matrix (ECM) protein (Sadeghi et al., 2021). It is constituted of up to 50% of the tumor mass (Mantovani et al., 2002). CMC is typically infiltrated by immune cells recruited by the tumor, which can create an immunosuppressive microenvironment that

promotes tumor growth by suppressing immune cells. Cancer-associated fibroblasts (CAFs), which are found in TME, are responsible for secreting various growth factors such as transforming growth factor, TGF-β, and platelet-derived growth factor (PDGF). TGF-β is believed to encourage the hindering of cytotoxic $CD8^+$ T cells from infiltrating the tumor, thereby promoting tumor survival (Lakins et al., 2018). Tumor-associated macrophages (TAMs) interact with tumor cells by secreting various immunosuppressive molecules such as cytokines, chemokines, and growth factors (e.g., TGF-β and vascular endothelial growth factor, VEGF), which can be differentiated into two types of macrophages: M1 macrophages (tumoricidal) and M2 macrophages (tumorigenic). TAMs then limit the effectiveness of the tumor-infiltrating lymphocytes (TILs) and suppress the NK cells' functions by promoting angiogenesis. Regulatory T cells (Tregs) are also recruited to the TME and play a role in tumor progression due to their immunosuppressive activity by secreting anti-inflammatory cytokines (e.g., IL-10). Moreover, TGF-β decreases the infiltration and activity of cytotoxic $CD8^+$ in TME (Li et al., 2020). Myeloid-derived suppressor cells (MDSCs) are one form of immune cells that function as immunosuppressive cells. These cells consist of immature monocytes and granulocytes which are released from the bone marrow into the blood during disease conditions including cancer. MDSCs assert their immunosuppressive actions using several mechanisms including the production of inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), and arginine, thus activating the release of prostaglandin E_2 (PGE_2) and arginase (ARG) levels (Kalinski and Talmadge, 2017). MDSCs can synergize T regulatory cells (Treg) and cause the downregulation of $CD8^+$ T cells. As a result, it is critical to understand how these factors interact, how this interaction promotes tumor progression or tumor regression, and the mechanisms involved to create effective therapeutic options by modulating the desired cell populations to be activated (Figure 1).

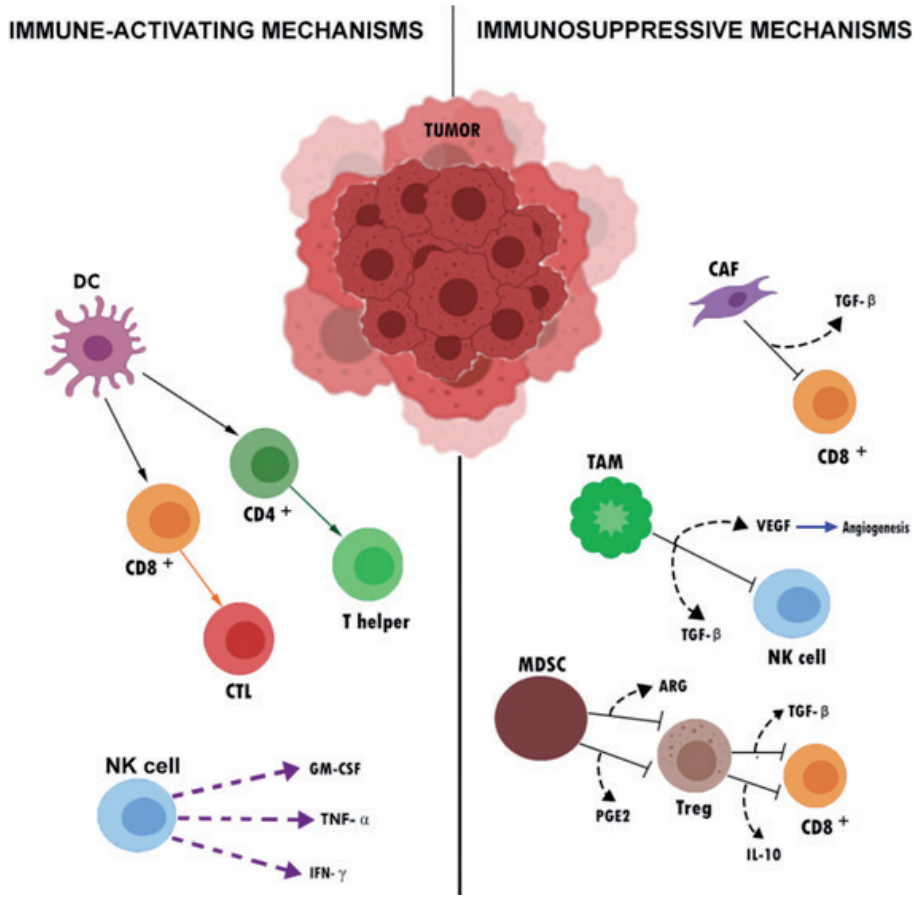

Figure 1. Schematic illustrations of different roles of TME-driven cell populations in canine mammary gland tumor progression. Cell populations within the TME are responsible for either activating or suppressing tumor growth. Dendritic (DC) and natural killer (NK) cells play immune-activating mechanisms to prevent tumor growth. CAF, TAM, and MDSC play an immune-suppressive mechanism by inhibiting $CD8^+$ and NK cells, respectively. By inhibiting NK cells, no secretion of proinflammatory cytokines including IFN-γ, TNF-α, and GM-CSF occurs; therefore, innate, and adaptive immune responses would not be activated. Meanwhile, inhibiting $CD8^+$ would cause no further activation of functionalized cytotoxic T lymphocytes (CTL) to attack and suppress tumor growth. Abbreviations: cytotoxic T lymphocytes (CTL), natural killer cell (NK cell), dendritic cell (DC), granulocytes macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), tumor-associated macrophage (TAM), cancer-associated fibroblast (CAF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), myeloid-derived suppressor cell (MDSC), arginase (ARG), prostaglandin E_2 (PGE2), regulatory T cell (Treg), interleukin-10 (IL-10)

Canine mammary carcinoma treatment

To date, surgical resection remains to be the mainstay choice of treatment (Gobello and Corrada, 2001; Lavalle et al., 2012). Lumpectomy and mastectomy are two types of surgical choices for the removal of mammary gland tumors, but the choice is often influenced by multiple criteria including the size of the mass, number of lesions, and condition of surrounding tissue. Lumpectomy is selected when the nodule is less than 0.5 cm firm, is superficial, and is a nonfixed nodule to the skin (Chang et al., 2005; Papazoglou et al., 2014). Mastectomy is divided into unilateral or bilateral and is often performed when there are multiple nodules present, which is indicated by the multiple glands of the chain involved. However, there is no difference in terms of relapse or prognosis rate between those approaches. The best approach is by removing the whole mass with wide surgical margins (Novosad, 2003). Then, this is followed by adjuvant therapy including chemotherapy or radiotherapy. In many reports, a better prognosis is achieved with a lower tendency of recurrence compared to surgical resection alone. Because malignant mammary carcinoma with lymphatic or vascular invasion has significant rates of recurrence and metastasis, surgical excision alone produces inadequate results (Gilbertson et al., 1983). It was reported that adjunct therapy with carboplatin and firocoxib after surgery improved the median survival as it reached up to 570 days compared to surgery alone, which was 63 days (Lavalle et al., 2012). In contrast, few studies reported the use of chemotherapy as adjuvant therapy did not affect a better prognosis (McNeill et al., 2009). Overall, surgical resection of the tumor remains the best approach in the treatment and prevention of CMC (Gobello and Corrada, 2001; Lavalle et al., 2012). These procedures include the removal of tumors of larger diameter, with more than one gland, along with the lymphatic system to avoid the risk of recurrence. However, the prognosis of the treatment depends on many factors: the age of the dog, tumor stage and size, and neutering status. This is in agreement with the studies by Moon et al. (2022) when they analyzed 60 cases of CMC to evaluate the prognosis after the tumor resection. The consensus information creates a new paradigm in the search for or improving the therapeutic modalities of CMC.

Adjuvant chemotherapy

Chemotherapy has long been used to treat dogs with CMC and people with breast cancer. Chemotherapy as an adjuvant therapy has improved overall survival rates significantly in the early stages of breast cancer, but it varies in the late stages. However, so far there is no standardized chemotherapy protocol for bitches with CMC (Levi et al., 2021). Chemotherapy is well-known as a painful treatment. Patient tolerability has often been a consideration in the planning of the chemotherapy cycle. Doxorubicin is the first line of chemo-drugs that is specially used for dogs with CMC. To date, there is no standard chemotherapy recommendation protocol for the treatment of CMC and the number of available prospective studies regarding this is quite limited (De Campos et al., 2018). However, many veterinary clinicians prefer the use of chemo drugs doxorubicin against CMC. Doxorubicin is in a class of anthracycline antibiotics and is derived from a secondary metabolite of a mutated strain of *Streptomyces peucetius* var. *caesius*. Doxorubicin effectively targets rapidly dividing cells, causing damage, therefore it is also referred to as a cytotoxic drug. Pharmacologically the drug prevents DNA and RNA synthesis by inhibiting topoisomerase II. It appears to attach to the nucleic acids of DNA via a specific insertion of anthracycline nuclei into the DNA of different types of cancer cells (Rawat et al., 2021). Foong et al. (2018) showed that doxorubicin was most lethal to canine mammary tumor cell lines compared to zerumbone and zerumbone-loaded nanostructured lipid carriers, based on an *in vitro* study. Simon et al. (2006) compared postoperative effects between doxorubicin and docetaxel in malignant canine mammary carcinoma. The results showed that doxorubicin improved the clinical outcome of dogs better than docetaxel, however, overall, both chemo drugs did not lead to an improved outcome for the dog population. To date, there is no standard protocol for chemotherapy against CMC, even though multiple chemotherapy protocols have been reported. Most protocols incorporate one or two cytotoxic drugs. For human breast cancer treatment, doxorubicin alone was well known for its several adverse effects and was not a preferred drug routinely used in women with breast cancer. Since doxorubicin is associated with many side effects including cardiotoxicity, leukopenia, nausea, vomiting, alopecia, and mucositis; polyethylene glycol doxorubicin with liposomal formulation (pegylated) was created to satisfy the safety purpose (Perez et al., 2002). It was the first FDA-approved nano-chemo drug (1995), commercially named Doxil® (Barenholz, 2012). Pegylated liposomes coated the doxorubicin resulting in a long half-life and good drug delivery to the targeted cancer cells. To date, limited information is available on clinically used pegylated liposomal doxorubicin in veterinary medicine for treating mammary carcinoma in canines. The use of Doxil® in CMC merits further investigation as cardiotoxicity is the most serious side effect limiting the use of doxorubicin in dogs (Zabielska-Koczywąs and Lechowski, 2017).

Adjuvant hormonal therapy

Hormonal therapies are well known to be used for the treatment of breast cancer in humans. The decision to choose hormonal therapy as an adjuvant is routinely based on histopathological assessment when there is evidence with regard to the presence of hormone receptors. Tamoxifen (Nolvadex) is an example of an estrogen receptor (ER) antagonist drug, which interferes with estrogen signaling by binding with the estrogen receptor in the mammary cancer cell. Apart from that, tamoxifen is also demonstrated to be a chemo-preventive medicine for high-risk breast cancer patients, especially a patient with

heredity issues, and the treatment is tolerated quite well (Joseph, 2002; Fisher et al., 2005). Studies by Liu et al. (2014) reported that tamoxifen can control ER-negative breast cancer through the inactivation of protein phosphatase 2A (PP 2A) and phospho-Akt (p-Akt) inhibition in the estrogen receptor, eventually stimulating apoptotic activity of tamoxifen and sensitizing the susceptibility of ER-negative breast cancer cells to tamoxifen. In theory, tamoxifen should work on dogs with ER-positive canine mammary gland tumors. Unfortunately, due to the unclear mechanism of tamoxifen, dogs are more sensitive to tamoxifen compared to a human, where their receptor levels are modulated easily (Gobello and Corrada, 2001). A study conducted by Tavares et al. (2010) reported that vulva edema and pyometra were diagnosed after around 90 days of tamoxifen treatment in a healthy bitch. Tamoxifen is responsive against CMC; however, ovariohysterectomy is highly recommended to overcome its adverse effects (Tavares et al., 2010). In addition, tamoxifen has an advantage as it can be given orally.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that inhibits cyclooxygenase production, thus preventing the activation of prostaglandins. There were two forms of cyclooxygenase enzyme: COX-1 and COX-2, which act as analgesic and antipyrexic. COX-1 is constitutively expressed in most tissues and regulates multiple physiological activities such as increasing gastrointestinal integrity and platelet aggregation, whereas COX-2 is induced by proinflammatory cascades and is overexpressed in a variety of cancers including mammary carcinoma (Üstün Alkan et al., 2012; Manikkan Dileepkumar et al., 2015). Queiroga et al. (2011) revealed that the COX-2 enzyme was expressed in CMC, and the high level of COX-2 enzymes was associated with a poor prognosis due to the stimulation of tumor angiogenesis, thus enhancing vascular density and tumor proliferation. Much attention is being focused on COX-2 inhibitors as an agent that can stop tumor progression. CMC in dogs has been demonstrated to overexpress COX-2.

In prior research, it was reported that among 84 samples of CMC, immunocytochemistry results showed that half of the samples were COX-2 positive (Brunelle et al., 2006). Overexpression of COX-2 in CMC is related to a high tumor histologic grade, a higher rate of tumor metastasis and recurrence, and a shorter patient survival time (Heller et al., 2005; Millanta et al., 2006). Therefore, owing to the reason that COX-2 plays a significant part in tumor progression, it may be considered beneficial to use NSAIDs as an adjunct treatment for CMC (Pang et al., 2014; Arenas et al., 2016).

Souza et al. (2009) reported that CMC dogs treated with piroxicam, which is a COX-2 inhibitor, showed a good clinical response to the treatment. Another study by Knottenbelt et al. (2006) showed the antiproliferation effects of piroxicam against canine mammary cells line. Treatment of CMC with piroxicam, either singly or following surgery and chemotherapy, can provide palliative care and improve the lifespan of dogs with CMC (Manikkan Dileepkumar et al., 2015). Another study conducted by Lavalle et al. (2012) discovered that adjuvant treatments (including those in conjunction with NSAIDs) for advanced CMC resulted in a statistically significant prolonged overall survival (OS) when compared to surgical treatment alone.

Another study by Pang et al. (2014) highlighted the antiproliferative and proapoptotic effects of mavacoxib against a few cancer cell lines including osteosarcoma, lymphoma, mast cell tumor, and hemangiosarcoma. This was supported by Hurst et al. (2019), who demonstrated the cytotoxic effects of mavacoxib against canine mammary carcinoma, urinary bladder carcinoma, and osteosarcoma.

Firocoxib inhibits COX-2, which initially suggests that there are clinical benefits against the tumor. Lavalle et al. (2012) discovered that firocoxib can prolong the overall survival and disease-free survival of dogs with grade III mammary tumors. However, the mode of action of firocoxib against CMC remains to be unclear. Several studies found that this was due to the association of COX-2 inhibitors in suppressing canine mammary tumor growth by inducing the apoptosis pathway (Rüegg et al., 2003; Wang et al., 2014).

Future directions translation from a human medicine perspective into veterinary medicine: rational of immunotherapies

Despite the availability of mastectomy for CMC, Nguyen and colleagues reported that the overall survival rate remains less than 2 years (Nguyen et al., 2018). Furthermore, the extent of chemo-drug response and potential relapse varies among dogs. So far, very limited studies on clinical trials for allowing the inclusion of dogs with CMC, however, there is a trend in exploring the efficacy of new targeted therapies for mammary cancers. Immunotherapy for CMC is an interesting and rapidly expanding field of study and application. With the advancement of techniques used to assess immune responses to a tumor, one can more reliably assess the clinical efficacy and safety of novel immunotherapy. There are better ways to predict responses, including a better understanding of tumor responses to immunotherapies, which may be delayed compared to conventional chemotherapy, radiation therapy, and surgery. On top of that, a better understanding of the disease pathology of veterinary patients has led to the use of spontaneous canine cancers as a model for cancer in humans, thus allowing for the testing of novel immunotherapies for small animal patients that will not only benefit them but benefit human cancer patients as well.

Immunotherapy strategies involving dogs have been investigated for decades. Canine cancers, like human cancers, have distinctive mutated tumor proteins that allow a patient's cancer-derived proteins to target and attack cancer cells (Weir et al., 2018). In human medicine,

monoclonal antibodies (mAbs) have been one of the convincing approaches for cancer immunotherapy. Some mAbs target specific molecules that interfere with signaling pathways (e.g., anti-HER-2). For example, when used in a combination with chemotherapy, the drugs trastuzumab, and pertuzumab have been demonstrated to enhance clinical outcomes among patients with HER-2-positive (HER-2^{+}) metastatic breast cancer (MBC), with the median overall survival (OS) increasing to 57 months (Swain et al., 2015; Costa et al., 2017).

Overexpressing of HER-2 protein in HER-2 type CMC can be the target for antibody-based therapies. Until now, HER-2 has dominated the scene of preclinical vaccine research in mammary carcinoma. Much preclinical research has shown that anti-HER-2 antibodies are effective against human breast cancer. The downregulation of oncogenic intracellular pathways triggered by HER-2 activation through homo- and heterodimerization in the cancer cell membrane has been attributed to the direct targeting of HER-2 by mAbs. These targeted antigens can be employed in the development of canine immunotherapy. Some mAbs work against immune checkpoint molecules such as PD-1 (atezolizumab, pembrolizumab, and nivolumab) and CTLA-4 (ipilimumab and tremelimumab), which can activate additional immunological responses such as antigen presentation and cytokine generation by releasing the cytotoxic activity of T cells (Valdivia et al., 2021).

Antibody-based therapies

Antibody-based immunotherapeutics is specific therapeutic agents that act based on the Fv region's affinity for antibody targeting as well as the Fc region's ability to interact with the host's immune system components (Harris and Drake, 2013). Monoclonal antibody drugs (mAbs) are a type of immunotherapeutic and they refer to antibodies that are produced against a single antigen by the B-lymphocytes. B-lymphocytes are activated when a foreign substance enters the body and antibody production occurs in acknowledgment of this antigen's epitope regions (Kimiz-Gebologlu et al., 2018).

The use of mAbs in human cancer treatment has expanded over the past year; however, veterinary medicine has yet to catch up. There are limited studies about the usage of mAbs for canine cancer disease and to date, there are none for CMC. Maekawa et al. (2017) conducted a pilot clinical study on the use of rat–dog chimeric mAbs to target PD-L1 in canine oral malignant melanoma. The dog with stage II disease showed an 81% reduction in the tumor burden. At present, several mAbs had been approved by FDA for human use against breast cancer such as trastuzumab, pertuzumab, pembrolizumab, and ramucirumab (Kimiz-Gebologlu et al., 2018; Weiner et al., 2010; Simpson and Caballero, 2014; Rue et al., 2015).

Due to the lack of cross-reactivity of some major human mAbs with the homologous canine target antigen, the development of mAbs has slowed in the veterinary industry. Unfortunately, to date, there are no versions of mAbs that are used in veterinary medicine, especially against CMC. Thus, future investigation of the use of these mAbs for CMC is warranted. At this time, studies on MABs have only been reported for canine T-cell lymphoma (Rodriguez et al., 2014) and canine hemangiosarcoma (Brown et al., 1985). Table 2 displays ongoing clinical trials of monoclonal antibody drugs (mAbs) in human breast cancer.

Table 2. Ongoing clinical trials of mAbs in human breast cancer

Agent	Phase	Clinical trials ID	Reference
MEDI4736 (a monoclonal antibody that targets PD-1)	II	NCT01693562	(National Library of Medicine, 2022)
MDX-11-5 (a human monoclonal IgG4 antibody targeting (PD-L1).	I	NCT00729664	(National Library of Medicine, 2022)
Atezolizumab (targeting PD-L1)	I	NCT01375842	(National Library of Medicine, 2022)
Trastuzumab (monoclonal anti-HER-2 protein antibody targeting)	III	NCT00045032	(National Library of Medicine, 2022)
Avelumab (targeting PD-L1)	I	NCT01772004	(National Library of Medicine, 2022)

Combination with immune checkpoint blocker

Now that mammary carcinoma is known as an immunogenic disease and is enriched in tumor-infiltrating lymphocytes (TILs) (Cimino-Matthews et al., 2016; Gatti-Mays et al., 2019), the reactivation of the immune system to destroy mammary tumors has emerged as a viable treatment option, where immune checkpoint inhibition is effective in both advanced and early-stage mammary carcinoma. The key to curing CMC successfully is suggested to be the use of multiple immunotherapies or a combination of therapies, as it is likely to be more effective. It creates better synergistic curative effects than single-target therapy due to the complex microenvironment of mammary cancer, hence leading to more rapid immunosurveillance and immunoediting phenomena, which are challenging.

To date, there is no guarantee of perfect protection or the eradication of CMC. Due to the immune escape mechanisms and tumor-mediated immunosuppression issues concerning mammary cancers (Steven and Seliger, 2018; Nelde et al., 2021), relapse may happen. In human oncology, combinatorial treatments are practiced. A single treatment may be effective in preventing relapse or for survival advantages with minimal side effects in cases of an early cancer diagnosis. On the other hand, combinatorial therapies may be able to successfully treat even advanced cancers while also overcoming immune escape mechanisms and tumor-mediated immunosuppression issues. There are many types of immune checkpoint block-

ade therapies such as programmed cell death-1 (PD-1) and CTLA-4 targeted antibodies. PD-1 is an inhibitory transmembrane protein expressed in T cells, B cells, and NK cells. PD-1 receptor interacts with its ligand (either PD-L1 or PD-L2) on cancer cells, hence resulting in immune checkpoint pathway activation. When PD-1 is overexpressed on T cells, B cells, and NKs cells, those immune cells are suppressed and deactivated, where they are hijacked by tumors (Kamphorst et al., 2017; Planes-Laine et al., 2019). Anti-PD-1 agents have shown promising results in a metastatic environment, while combination strategies tend to induce more responses (Weiner et al., 2010). The tumor initiation, progression, metastasis, and development of immune response in humans closely resemble that of dog disease. Although PD-1 and CTLA-4 had been detected in CMC (Shosu et al., 2016; Ariyarathna et al., 2020), no attempts to employ immune checkpoint blockade agents against CMC have been made to date. Regarding the potential of blocking PD-1, only one type of canine cancer was reported; however, it was for oral malignant carcinoma (Igase et al., 2020). Other studies from Son et al. (2014) and Foy et al. (2016) reported significant induction of cytotoxic T lymphocytes (CTLs), which resulted from CTLA-4 blockage, hence eliciting antitumor immunity in a murine cancer model. Thus far, no combinatorial studies have been made for CMC compared to human and murine studies, hence suggesting that it is possible to be translated into canine cancer. The study of pembrolizumab plus chemotherapy as a first-line treatment of triple-negative breast cancer is ongoing and is currently in phase 3 clinical trials. Another combination of immune checkpoint inhibitors is the combination of atezolizumab with chemo drugs, which shows remarkable results and is in the clinical trial stage (Wein et al., 2018). In murine models, the blocking of PD-1/PD-L1 was shown to promote T cell-mediated antitumor immune activity (Hirano et al., 2005). Like human and their murine counterparts, canine patients may also benefit from these findings. These findings provide a convincing idea that anti-PD-1/PD-L1 agents may act synergistically to induce a stronger immune response against the tumor. Table 3 displays ongoing clinical trials of drugs combined with a checkpoint inhibitor in human breast cancer.

Adoptive cell therapy

Adoptive cell therapy (ACT) is a type of immunotherapy in which a patient's T cells lymphocytes (such as $CD8^+$ cells, $CD4^+$ helper cells, TILs, NKs, etc.) have been manipulated and utilized in cancer patients to obtain immunity against human breast cancer (Li et al., 2021). Cytokine-induced killer (CIK) cells are an innovative form of immunotherapy that is made by cultivating peripheral blood mononuclear cells (PBMCs) with IFN-γ, anti-CD3 antibody and IL-2 to produce T effector cells (Verneris et al., 2002). CIK cells expressed natural killer group 2 member D (NKG2D) receptor and blocked lymphocyte function-associated antigen-1 (LFA-1) and intracellular cell adhesion molecule-1 (ICAM-1) thus releasing granzyme and perforin which were delivered to the surface of target cells and promoting cytolytic killing mode (Cullen et al., 2010; Pievani et al., 2011). CIK cell therapy was found possessed synergistic effects and has shown tremendous improvement against cancer preclinically and clinically when in combination with standard therapies (Anguille et al., 2015). Pan et al. (2014) investigated the effectiveness of the combination of CIK infusion with either chemotherapy or radiotherapy in 90 patients with post-mastectomy triple-negative breast cancer (TNBC). The result showed significantly higher disease-free survival (DFS) and overall survival (OS) rates in the CIK treatment group compared with those who received treatment with standard chemotherapy or radiotherapy alone.

Table 3. Ongoing clinical trials of drugs combined with a checkpoint inhibitor in human breast cancer

Agent	Phase	Clinical trials ID	Reference
Nivolumab (targeting PD-1) ± ipilimumab (targeting CTLA4)	I/II	NCT01928394	(National Library of Medicine, 2022)
Lirilumab (target killer-cell immunoglobulin-like receptors) + nivolumab (targeting PD-1)	I	NCT01714739	(National Library of Medicine, 2022)
Imprime PGG + pembrolizumab	II	NCT02981303	(National Library of Medicine, 2022)
CFI-400945 + durvalumab (targeting PD-L1)	II	NCT04176848	(National Library of Medicine, 2022)
Palbociclib (inhibitor of CDK4 and CDK6) + avelumab (targeting PD-L1)	I	NCT04360941	(National Library of Medicine, 2022)

Canine mammary gland carcinoma vaccine

Recent advancements in many fields have reignited interest in the production of prophylactic cancer vaccines and have paved the way for success. These breakthroughs have been made in target selection, vaccine technology, and strategies for reversing cancer's immunosuppressive mechanisms. Target properties that yield high efficiency and sufficient immunogenicity to influence clinical outcomes have been discovered in studies on the targeting of tumor antigens. Several tumor-associated antigens (TAAs), which are natural host proteins that are abnormally expressed in cancer cells, are good immunotherapy targets. A vaccine is a form of immunotherapy that enhances immune recognition and prevents disease through cellular and humoral responses. The discovery of TAAs and tumor-specific antigens (TSAs) has allowed for the development of techniques to specifically target neoplasms immunologically. In human breast cancer, the antigens HER-2/neu (HER-2) and mucin-1 (MUC1) are the most well-studied in breast cancer. MUC1 is

expressed in the great majority of breast tumors with altered glycosylation, whereas HER-2 is overexpressed in 20–40% of breast cancers (Ernst and Anderson, 2015). Generally, cancer vaccines stimulate $CD4^+$ and $CD8^+$ T cell responses against (TAAs) or TSAs, before finally developing memory with regards to the tumor. CMC is one of the best-known homologous models of human breast cancer, which shares many similarities such as frequent oncogene HER-2/neu activation and p53 expressions (Ahern et al., 1996; DeInnocentes et al., 2006). Tumors express specific tumor-associated antigens (TAAs) as a target for the immune system mediated by CTLs (Coulie et al., 2014). The presence of multiple tumor-specific antigens on every tumor offers a justification for immunotherapy techniques that are now being investigated in human medicine.

Several works had demonstrated the development of the CMC vaccine. Bird et al. (2019) studied the development of canine mammary carcinoma cell–dendritic cell fusions as a vaccine. Approximately 30 dogs were vaccinated, and the results showed that the vaccine-induced significant enhancement of CTLs activity and serum immunity in normal healthy dogs with no immune-related adverse effects. Immunization with a vaccine based on DCs can result in generating powerful CTLs responses against cancer (Perez and De Palma, 2019). The first FDA-approved personalized vaccine for prostate cancer in humans, Sipuleucel-T, was a type of dendritic cell vaccine (Mastelic-Gavillet et al., 2019).

In another research, Peruzzi et al. (2010) developed a plasmid DNA electroporation (DNA-EP) telomerase reverse transcriptase (TERT) peptide vaccine against HER-2/neu positive CMGT. The results revealed that the vaccine induced a high level of $CD8^+$ immune response and IFN-γ, which were expected to play a major role through their antitumor effects. Weir et al. (2018) performed Phase-1 clinical trial on an autologous cancer vaccine containing Advax™ adjuvant against various canine tumors including CMC in dogs. Even though controlled clinical trials with increased sample size are highly recommended for future research, early data showed the clinical benefits of the autologous vaccine against CMC in terms of improving survivability and quality of life. Gabai et al. (2014) conducted a pilot study on the p62 DNA vaccine involving 7 dogs with canine mammary gland tumors. The vaccine was found to be effective and led to tumor regression.

In line with breast cancer in humans, steroid-hormone receptor (ER/PR) expressions are common in canine mammary carcinoma and these receptors play an important role in mammary tumor development. Furthermore, other characteristics of dogs, such as p53 overexpression and mutations, HER-2 overexpression, and the tumor's immune milieu, are substantially equivalent and show similar clinical correlations to those of humans. Based on the pathophysiological similarities between canine and human mammary gland carcinomas, dog cancer patients can serve as suitable model subjects (Carvalho et al., 2014; Fazekas et al., 2016).

HER-2/neu, which is also known as ErbB2, NEU, and CD34, is a human epidermal growth factor receptor 2 and is a component of transmembrane glycoprotein that is overexpressed in approximately 20–40% of primary breast carcinomas for tyrosine kinase activity (Ernst and Anderson, 2015; Ressel et al., 2013). A recent study found that canine mammary gland cancers had a similar HER-2/neu overexpression rate to their human disease counterparts (Carvalho et al., 2014; Ressel et al., 2013). Shariat and colleagues conjugated liposome with P5, which is an HER-2/neu synthetic peptide derived from TAAs (Shariat et al., 2014). The results showed that the immunized mice exhibited higher IFN-γ production by the $CD8^+$ T cells intracellularly, which represented a higher CTLs response. The results suggest that the vaccine can be further developed against the HER-2 type of mammary carcinoma (Shariat et al., 2014). Another study conducted by Razazan et al. (2017) conjugated nanoliposomes with GP2, which is an HER-2/neu-derived peptide that acts as an effective vaccine against breast cancer in mice models. The results showed that the vaccines did not just induce a high level of IFN-γ, $CD8^+$ cells, and CTL response, but they were also able to significantly delay the tumor growth in the vaccinated group.

Farzad et al. (2019) demonstrated that another peptide, P435 HER-2-derived peptide, conjugated to liposomes that could induce robust CTLs reactions, therefore improving cancer prognosis in the TUBO murine mammary cancer model. The results demonstrated that the vaccinated mice exhibited the lowest tumor size and the longest survival time in the TUBO murine mammary cancer model. Targeting HER-2 appears to be a wise approach to the deregulation of multiple signaling cascades that foster an oncogenesis pathway through HER-2/neu-targeted vaccines. Moreover, it was demonstrated that the targeting of HER-2/neu with trastuzumab led to the growth inhibition of canine tumor cells, indicating similar biology in canine mammary carcinomas as that of the HER-2/neu system in human patients (Muhammadnejad et al., 2012; Singer et al., 2012).

To date, the cancer vaccine approach and studies in veterinary medicine are still limited compared to human medicine. The development of vaccines for the prevention of human cervical cancer has been the most successful in humans. The future of immunotherapy appears to be quite promising if it translates to veterinary patients.

Conclusions

Canine and human breast cancers are similar on many levels. The development of disease composed of malignant mammary carcinomas in patients of both species demonstrates similar age associations, as well as other risk factors that correlate with tumorigenesis. In addition, tumors in both species develop spontaneously in the context of the tumor microenvironment and immune system. Adopting immunotherapy techniques including mAbs, immune checkpoint blockers, and vaccine approaches that incorporate antigens as targets in human

medicine allows the translation of technologies between species, thus creating a new paradigm in the search for canine veterinary medicine. Immunotherapies have the potential to become an additional therapeutic option for CMC as a single treatment or incorporate into conventional treatment modalities. Therefore, further research and investigation will be needed to develop better treatment options and clinical benefits for canine mammary gland carcinoma patients.

Author contributions

Authors shared equally in this work. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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