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Review

Exosomes as Biomarkers of Human and Feline Mammary Tumours; A Comparative Medicine Approach to Unravelling the Aggressiveness of TNBC

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ABSTRACT

Comparative oncology is defined as the discipline that integrates naturally occurring cancers seen in veterinary medicine, into more general studies of cancer biology and therapy in humans, including the study of cancer-pathogenesis and new cancer treatments. While experimental studies in mice and rodents offer several advantages, including a wealth of genetic information, reduced variation and short generation intervals, their relevance in cancer biology is somewhat limited. Toward this end, as the biomedical research community works to make the promise of precision medicine a reality, more efficient animal cohort studies are critical. Like humans, *companion animals* such as cats and dogs living in family homes, are exposed to environmental factors that may influence the development of disease. Furthermore, it has been shown that the basic biochemical and physiological processes of *companion animals* more closely resemble humans compared to rodents. Research has demonstrated that female domestic cats (*Felis catus*) may represent a comparative model for investigation of mammary carcinogenesis, and in particular, Triple Negative Breast Cancer (TNBC). TNBC is a subtype of breast cancer that typically lacks the expression of the oestrogen receptor (ER), progesterone receptor (PR), and does not overexpress the human epidermal growth factor receptor 2 (HER2).

An exciting and rapidly expanding area in cancer biology is the study of exosomes. Exosomes are nanoparticles released from cells and have been found in biological fluids of humans, domestic cats and dogs. In addition to their role as biomarkers, exosomes are implicated in the pathogenesis of certain diseases, including cancer. This review explores the current understanding of exosome biology in human TNBC, and of the potential benefits of comparative research in naturally-occurring mammary tumours in companion animals.

1. Triple Negative Breast cancer (TNBC)

1.1. What is Triple Negative Breast Cancer (TNBC)

Triple negative breast cancer (TNBC) is a subtype of breast cancer that typically lacks the expression of the oestrogen receptor (ER), progesterone receptor (PR), and does not overexpress the Human Epidermal Growth Factor Receptor 2 (HER2). Consequently, treatments that have been developed to target these receptors are not effective in this form of breast cancer. TNBC accounts for between 10 and 15% of all breast cancer subtypes [35]. TNBC shares some molecular and biological characteristics with so called '*basal like*" breast cancer, but these tumour subtypes are not identical [35]. In general, TNBC is an aggressive form of cancer with a higher relapse rate and metastatic potential, than other types of breast cancer [2,101], although increasingly, low grade, more indolent forms, are recognised [40]. TNBC most commonly metastasises to the lung, liver and brain [101]. Metastasis in breast cancer is a multistep process involving dynamic interactions between tumour and stromal cells, and the host immune microenvironment, ultimately resulting in the detachment and movement of primary tumour cells to secondary sites [3]. Despite best efforts to develop personalised therapies for women with TNBC, fewer than 30% of patients with metastatic TNBC are alive at five years following diagnosis, and almost all unfortunately will die as a result of their cancer [2].

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1.2. Genomic TNBC Classification

Understanding the complexity of TNBC is a focus of many research efforts, including the Cancer Genome. Several emerging research efforts have aimed to increase the understanding of the complexity of TNBC including the Cancer Genome Atlas Research Network, who have analysed primary breast cancers using platforms such as DNA methylation, exon sequencing and reverse-phase protein arrays (Cancer Genome Atlas [21]). This work has shown that the most frequent *loss-of-function* alterations in TNBC, involves genes associated with DNA damage repair, including loss of the tumour suppressor genes TP53, RB1 and BRCA1 functions. Aberrant activity of PI3K signalling pathways, has also been frequently reported (Cancer Genome Atlas Research [21]). Further information to support the heterogeneity of TNBC comes from a study by Shah et al. [86], who sequenced and analysed TNBCs from 100 women at the time of diagnosis. High rates of somatic mutations in the TP53 gene were found; mutations that result in an altered p53 protein that is less able to control cell proliferation. However, 12% of tumours did not have any recorded somatic mutations in these *so-called* driver genes [86].

Gene expression analyses have demonstrated the diversity of intrinsic subtypes of TNBC, and have partially helped to decipher the complexity of TNBC heterogeneity. Approximately 70% of TNBC cells are reported to be "*basal-like*" [79]. Similarly, not all "*basal-like*" tumours are "*triple negative*" by immunohistochemistry (IHC) [79]. In other words, although "*triple-negative*" and "*basal-like*" breast cancers share similar morphological features, these terms are not interchangeable. The term triple negative is formed on the basis of clinical assays for ER, PR and HER2, whereas the term "*basal-like"* is based on microarray gene expression analyses. The majority of metaplastic and medullary-like cancers which are typically TNBCs, also show a "*basal-like"* phenotype, which is rarely found in other types of breast cancer [13]. Significant efforts in genome-wide gene expression profiling analyses have improved our understanding of the diversity of TNBC, with at least six different subtypes being described by Lehmann et al. [58] namely *basal-like* 1, *basal-like* 2, immunomodulatory, mesenchymal, mesenchymal stem-like and luminal androgen receptor positive. These subtypes have been reproduced and pharmacologically targeted in breast cancer cell lines, as evidence that they can potentially inform therapy selection. However, for patients, very little is known about the factors driving these distinct molecular events or how to treat them effectively using targeted therapies. Therefore, it has been argued that the relevance of classifying TNBC in this way is somewhat trivial. However, in reality, this classification is a significant advance and will form the basis of future treatments [8,58]. More extensive research is needed to clarify the potential impact of this classification strategy on TNBC treatment decisions.

1.3. Histology

According to a study of 19,900 TNBC patients [60], most patients with TNBC have a histological diagnosis of invasive ductal carcinoma, no *special type* [23] (Fig. 1). Other histological subtypes with a triple negative profile include carcinoma with medullary features, metaplastic carcinoma, apocrine carcinoma, pleomorphic invasive lobular carcinoma, adenoid cystic carcinoma and microglandular carcinoma [16,31]. The histological heterogeneity is reflected in clinical outcome, including survival rates [60].

In general, "*Triple Negative*" and "*basal-like*" tumours are characterised by aggressive histological features including high tumour grade, a high degree of mitotic activity and necrosis. Tumours are frequently large and tumour infiltrating lymphocytes (TILs) may be prominent [9,53]. In addition, a further contribution to the heterogeneity of TNBC, is that low grade TNBCs are now recognised, for example adenoid cystic carcinoma and secretory carcinoma [40].

At the immunohistochemical (IHC) level, "*basal-like*" breast cancers are characterised by the expression of proteins normally found in the basal/myoepithelial cells of the normal breast, including high molecular weight (*basal*) cytokeratins (CK5/6, CK14 and CK17) (Fig. 2). Therefore, the term "*basal-like"* stems from the similarity between the IHC and molecular profile of these tumours, and that of basal/myoepithelial cells of the normal breast [77]. This *"basal-like*" subtype is typically associated with a poor clinical outcome, most likely reflecting its high proliferative capacity, high grade, and the lack of targeted systemic therapy, since "*basal-like"* tumours express a low level of ER and do not overexpress HER2 [88]. The poor prognosis associated with the "*basal-like*" phenotype of breast cancer may be due to lack of ER expression and cyclin E positivity, rather than to expression of CK5/6 or CK17 expression [78].

1.4. Treatments: Surgery and Chemotherapy

The mainstay treatment for patients with TNBC, remains cytotoxic chemotherapy, such as anthracycline and taxane-based chemotherapy. Specifically, in addition to locoregional treatment, the standard care for TNBC is combination chemotherapy, most usually and traditionally, doxorubicin in combination with carboplatin or cyclophosphamide [55]. Thus far, no appropriate molecular targets have been identified for patients with TNBC, and they therefore do not benefit from targeted therapies such as endocrine therapy that are fuelling the personalised medicine era [37]. For the most part, the lack of targeted therapies available for TNBC is due to the innate heterogeneity of TNBC itself [59].

Neoadjuvant chemotherapy has been historically used to downstage non-resectable tumours for better loco-regional control and higher conservative surgery rate [80]. After receiving neoadjuvant chemotherapy, about 30% of patients with TNBC present with a pathological complete response (pCR). Pathological complete response is defined as the absence of residual invasive cancer in the resected breast specimen and all regional lymph nodes sampled following completion of neoadjuvant chemotherapy [80]. After neoadjuvant therapy, achieving a pCR improves the outcome for TNBC patients to the point that their disease free survival and overall survival, are similar to patients with less aggressive, non-triple negative tumours [29]. However, TNBC patients with residual disease following neoadjuvant and adjuvant chemotherapy, typically have a worse survival and prognosis than those non-triple negative presentations. Some are of the opinion, that pCR may therefore be considered a potential surrogate marker for survival in TNBC [22,29].

Breast-conserving surgery (BCS) including radiotherapy (RT), has been found to provide at least equivalent prognosis to mastectomy in early stage breast cancer [93]. However, studies on women with TNBC are relatively scarce [93], with the outcomes associated with BCS and mastectomy remaining controversial. Specifically, a study by Chen et al. [26], used the Surveillance, Epidemiology, and End Results (SEER) database, to enrol 11,514 female TNBC cases who were diagnosed during the period of 2010–2013. Patients were subdivided into two groups; those who received BCS and RT, and those that received a mastectomy. When the patients were stratified according to age, histology grade, TNM stage, tumour size and lymph node status, the majority of patients who received BCS together with radiotherapy, demonstrated a better survival (both overall survival and breast cancer survival), compared to the mastectomy group alone, therefore providing evidence for surgeons in favour of BCS for women with TNBC.

Radiation therapy has been shown to be useful for the management of TNBC. The indications for radiotherapy for breast cancer are mainly based on TNM staging. Radiation therapy of the chest wall after mastectomy, and the regional area, as well as after breast conserving therapy is a relatively common treatment approach for TNBC [98]. A cohort study by Abdulkarim et al. [1], involving 768 patients with early stage TNBC, investigated whether postoperative radiotherapy can minimise the risk of local recurrence of TNBC. This study showed that

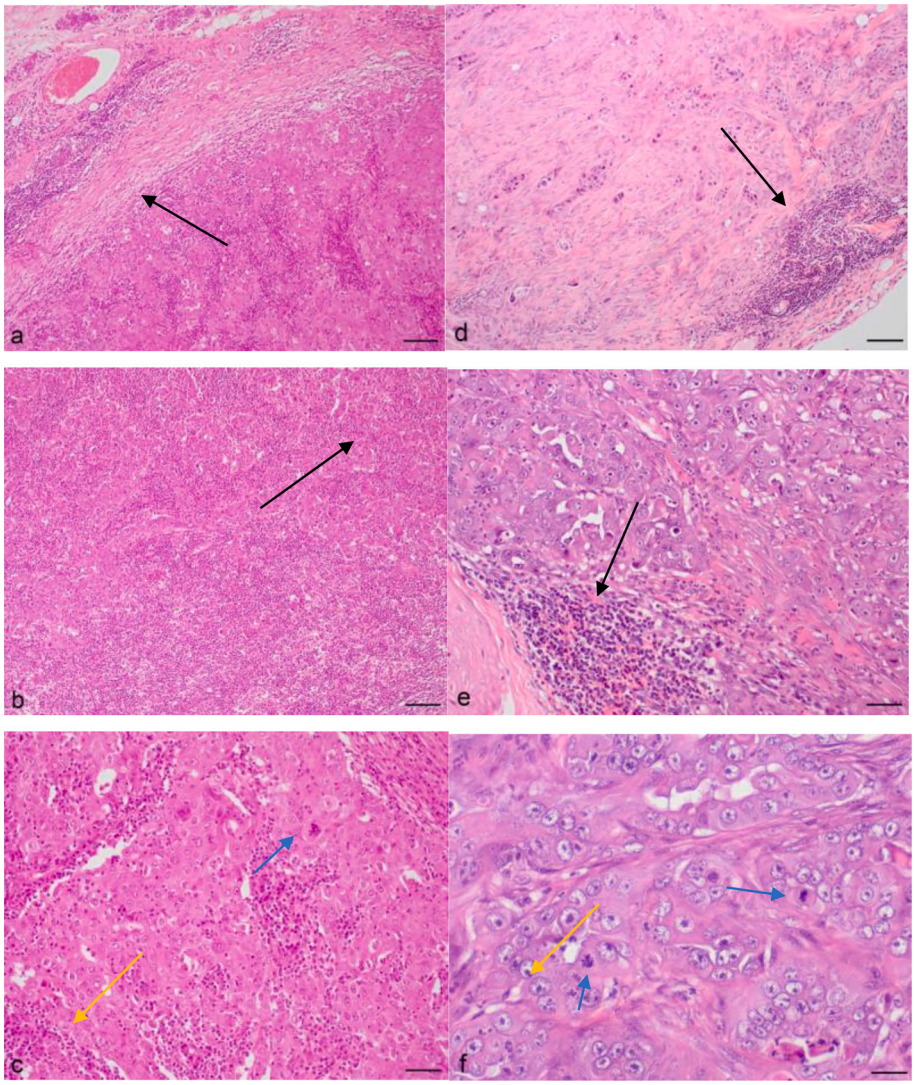

Fig. 1. Haematoxylin and eosin (h & e) stained tumour sections comparing the histological features of a human high-grade invasive breast carcinoma (a, b, c) compared to a feline high grade mammary adenocarcinoma (d, e, f). **1a** demonstrates the circumscribed outline of the carcinoma and some lymphocytes at the periphery of the tumour. **1b** shows the solid arrangement of the tumour cells with intervening prominent lymphocytes (see arrow). **1c** shows marked nuclear pleomorphism (yellow arrow) and abnormal mitotic figures (blue arrow).**1d** and **1e**, show prominent lymphocytes at the periphery of the tumour. **1f** demonstrates marked nuclear pleomorphism (yellow arrow) and abnormal mitotic activity (blue arrow). **1a, 1b** and **1c** have scale bars representing 100μm diameter (**1a** and **1b**) and 50μm (**1c**). **1d**, **1e** and **1f** have scale bars representing 100 μm, 50 μm and 25 μm respectively. Feline images were taken from a 5-year-old female neutered domestic short haired cat, while human images were taken from an adult female with a diagnosis of TNBC.

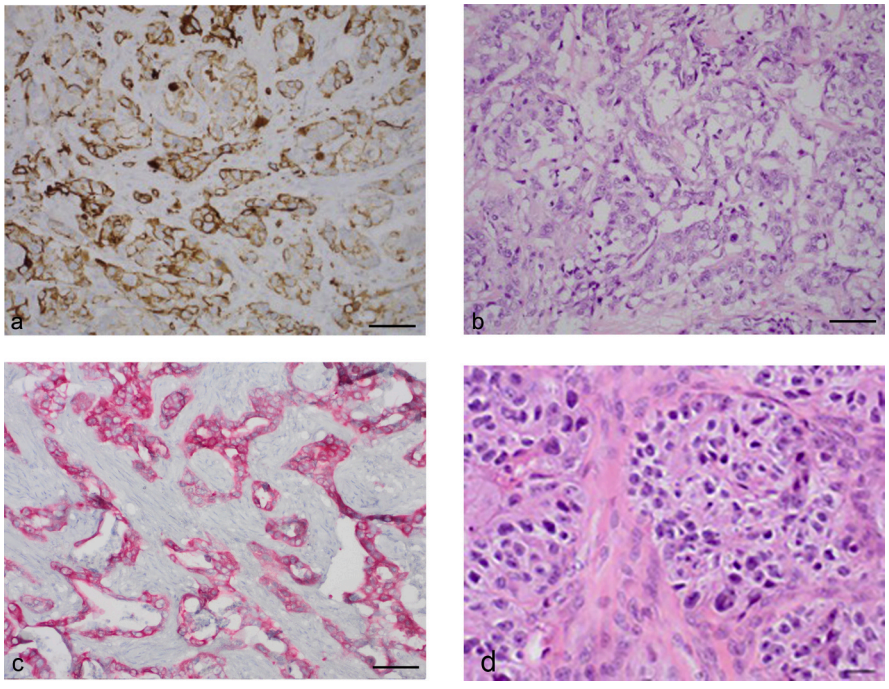

Fig. 2. Immunohistochemical staining of cytokeratin 5/ 6 showing "*basal-like*" positive signatures in both human (a) and feline mammary adenocarcinoma (c) with paired haematoxylin & eosin stained sections (b & d). **2a** represents positive staining for cytokeratin 5/6 in human breast cancer with matched haematoxylin & eosin stained section (**2b**). **2c** represents positive staining for cytokeratin 5/6 of a feline high grade mammary adenocarcinoma (FMA) with matched haematoxylin & eosin stained section. This feline also had multiple lymph node metastases and had marked desmoplasia both within the mammary gland tumour and within the lymph nodes. Size bars represent 50μm for 2a and 2b. Size bars represent 25 μm for **2c** and **2d.** Feline images were taken from a 14 year old female neutered domestic short haired cat, while human images were taken from an adult female with a diagnosis of TNBC.

women treated with radiation therapy had significantly increased survival when compared to those women who did not receive radiation therapy, highlighting the importance of radiotherapy within the TNBC treatment regimen [1]. In addition, in relation to radiotherapy post-mastectomy, it has been shown that radiotherapy can improve the outcome for TNBC. A prospective randomised trial by Wang et al. [96] examined the postoperative treatment regimen of women following radical mastectomy, demonstrating that the 5-year overall survival rate of patients treated with radiotherapy, was 11.6% higher than that of the non-radiotherapy group, supporting the post-operative radiation for women with TNBC [96].

1.5. Clinical Trials and the Unmet Clinical Need in TNBC

Patients diagnosed with TNBC represent a substantial *unmet clinical need*. The sheer characterisation of TNBC as ER, PR and HER2 negative, represents one of the most prevalent clinical challenges for these patients, because they cannot be treated with endocrine therapies or other readily available targeted therapies (for example Herceptin/Trastuzumab). The majority of clinical trials [28] in TNBC, are focused on varying combinations of cytotoxic chemotherapy in order to maximise the disease free survival period [48]. Additionally, in comparison to other breast cancers, TNBC has been associated with an increased metastatic potential, with a shorter median time to relapse and death. One of the most important goals in TNBC research is the identification of markers to predict the location of metastasis and to predict treatment response [48].

Based on this profound *unmet clinical need*, Synnott et al. [91] have suggested that as the tumour suppressor gene TP53 is mutated in approximately 80% of TNBC cases [75], this may constitute a potential biomarker for TNBC patients. COTI-2 is a third generation thiosemicarbazone derivative, which acts by reactivating mutant p53 to a wildtype form. It has been developed for high efficacy and low toxicity as a potential targeted therapy for TNBC patients by acting on mutated p53. However, it is also capable of acting "*independently of p53, by inhibition of the mTOR pathway and activation of AMPK*" [61]. COTI-2 is currently showing great potential as it undergoes phase I clinical trials for the treatment of gynaecological and other cancers [46]. Synnott et al. [91] showed the anti-proliferative effects of COTI-2 in response to mutant p53 in TNBC, and also supported previous reports, demonstrating that, in addition to inhibiting the growth of mutant p53 cell lines, COTI-2 also inhibited the proliferation of p53 wildtype cells [18,32]. This may be as a direct result of mTOR pathway inhibition [91]. Interestingly, when COTI-2 was combined with traditional cytotoxic chemotherapy, such as doxorubicin or carboplatin, synergistic growth inhibition of mutant p53 cell lines was observed. While only at an early clinical trial phase, COTI-2 represents one of the first and only targeted treatments for TNBC patients [91].

2. Comparative Oncology

2.1. The Need for Alternatives to Mouse Models in Cancer Research

Despite an improved understanding of cancer molecular biology, immune landscapes and advances in cytotoxic, biologic, and immunologic anti-cancer therapeutics, cancer remains a leading cause of death in both animals and humans worldwide. While experimental studies in mice and rodents offer several advantages, including a wealth of genetic information, reduced variation, short generation intervals, ease of maintenance and handling at a more affordable cost, the lack of genetic diversity, shared environments, lifespan and naturally-occurring tumours, constitute the importance of *companion animal* patient cohorts. According to a report published by the National Academies of Sciences in 2015, a mere 11% of anti-neoplastic therapies that demonstrate efficacy in murine models have been approved for human use, representing a high attrition rate for emerging therapies as well as a need for more advanced and more relevant animal studies [42]. It is therefore clear that there are several weaknesses associated with the use of laboratory animals as models in cancer research. For example, humans are 3000 times larger than mice, they also live up to 50 times longer undergoing about 105 more cell divisions in a single lifetime [81]. Furthermore, it has also been proven that the microenvironment of tumours in genetically engineered mouse models is significantly different in a number of ways to that of human cancers. This differing microenvironment along with the genetically bred homozygosity of mice, often results in overly favourable predictive responses to chemotherapy, radiation therapy and immunotherapy [47].

Although murine studies have been invaluable, and will remain a critical first step of preclinical research for cancer biology and therapeutics, their shortcomings and the need for additional, be it more reliable animal models are being increasingly recognised. With better models/cohorts that recapitulate human conditions emerging, it is possible that fewer failures may be observed in human clinical trials. In addition, better models may also shorten the pre-clinical and clinical trial phases [84]. Toward this end, as the biomedical research community works to make the promise of precision medicine a reality, novel animal models of *naturally-occurring cancer* may play a crucial role in bridging the gap between fundamental diagnostics, therapeutics and human clinical trials.

2.2. Comparative Oncology: A Definition

The term "*Comparative Oncology*" has been defined as the discipline that integrates the naturally occurring cancers seen in veterinary patients into more general studies of cancer biology and therapy, including the study of cancer-pathogenesis and new treatments [92]. In 1896 during his valedictory address at McGill University, Wesley Mills recognised the importance of the discipline stating that "*Comparative medicine is the medicine of the future, and the sooner it is realised, the better, for man and beast*" [69]. More recently, Garden et al. [39] described the "far-reaching benefit of comparative oncology" for both animals and humans with a diagnosis of cancer.

Although a number of veterinary species including the cat, horse, pig and ferret, have demonstrated comparative potential, the majority of research efforts thus far have focused on the dog [102]This focus is due to the strong anatomical and physiological similarity between dogs and humans. While extremely beneficial for both cohorts, a shift in focus to other animals such as cats and pigs has the potential to benefit other disease areas. Cancer is a major cause of morbidity and mortality in animals [4], with reports indicating that cancer kills 40–50% of canines over the age of 10 years [4,94].

2.3. Advantages of Studying Spontaneously Occurring Cancer in Companion Animals

Precision medicine is simply defined as the right treatment for the right patient, at the right time. This therefore demands highly relevant translational models to recapitulate disease [84]. To date, cancer research has been limited by the lack of genotypically and anatomically relevant non-rodent animal studies. In theory, an ideal animal for comparative oncology should **(i)** mimic disease on a molecular basis, **(ii)** derive from a relevant tissue type, **(iii)** be reliable and predictable, **(iv)** manifest survival differences, **(v)** allow for accurate treatment assessment, **(vi)** be readily imaged and **(vii)** occur in a similar background to the human disease [6]. Investigating spontaneously occurring cancers in *companion animals* is an underutilised clinical resource that can play a crucial role in bridging the gap between fundamental diagnostics and human clinical trials. When compared to murine/rodent models, there are numerous advantages of spontaneously occurring disease in animal studies that increase their clinical fidelity, making them a potentially useful tool in cancer research and the precision medicine era. Spontaneous disease in companion animals such as cats

or dogs, are capable of closely recapitulating human cancer and associated comorbidities. This therefore, potentially provides an avenue for the analysis of both human and animal cancers [84]. The natural occurrence and history of cancer in animals and humans permits the rapid study of DNA damage and epigenetic alterations that accumulate over time underpinning resultant tumour development [43]. Some animals are said to exhibit a "*compressed pathology*", whereby cancer progression, development of metastasis and response to chemotherapy is seen at a more rapid rate, compared to humans with a similar cancer type.

As humans and animals share many diseases, it is not surprising that they often share similar genetic mutations. A commonly used example of this similarity is canine X-linked haemophilia A, which is caused by a gene inversion in factor VIII, the same as in humans [62]. Despite large genetic variations due to outbreeding of some animal species, selective breeding has led to an increased prevalence of particular cancers in dogs and cats. The study of cancer genomics in animals offers the opportunity to identify new gene associations currently not recognised in human disease which could translate into the identification of novel targeted therapies. Similar to humans, cats and dogs living in family homes as pets are also exposed to the same lifestyle risk factors and external environmental factors such as toxins and carcinogens. Similar to humans, these factors often influence the development of certain diseases. Passive smoking has been shown to increase the risk of the development of malignant lymphoma [11] and oral squamous cell carcinoma [12] in cats, while obesity increases the risk of development of diabetes mellitus in domestic cats [25]. Similarities are also noted in therapeutics, where the efficacy of certain chemotherapeutic agents and/or other hormonal therapies is similar in animals as in man [76]. Specifically, as an example, CHOP-based (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) protocols are the mainstay of treatment for certain lymphomas in man and in dogs, and result in similar response and remission rates [136] Importantly, the heterogeneity of diseases seen in veterinary medicine closely models that of human patients. Specifically, it has been shown by Parker et al. [76], that the basic biochemical and physiological processes of dogs more closely resembles human beings compared to rodents [76].

2.4. Canine Breast Cancer

Canine cancer is a serious clinical issue in veterinary medicine. It is estimated that one in four dogs greater than two years of age will die from some sort of cancer, with certain breeds such as Bernese mountain dog, Irish wolfhound, flat-coated retriever and Saint Bernard, over represented in terms of cancer incidence and mortality [33,73,92]. Interestingly, the risk of tumour development increases depending on the time of ovariohysterectomy (spaying/neutering) [10]. According to Beauvais, Cardwell and Brodbelt, if a dog is spayed before her first ovarian cycle, the risk of tumour development is 0.5%. However, if spayed just after or any time later, the risk increases to 8%. Approximately 50% of canine mammary tumours are malignant [10].

Mammary tumours are the second most common neoplasia in dogs, typically occurring in females between the ages of 8–10 years [87]. Canine Mammary Tumours (CMT) are highly similar to human breast cancers at the clinical level [121]. Therefore, it is not surprising that human biomarkers of breast cancer are also detectable in cases of CMT [121] [52]. The incidence of cancer in dogs has increased steadily [73]. This may be due to an increase in the population of dogs at risk, and/or the awareness and interest of pet owners. The improved general health of pets as a result of vaccines, better nutrition and better, more accessible veterinary care are allowing dogs to live longer, which may indeed be contributing to the increased incidence of age-related diseases such as cancer observed in these patients [73]. Thus far, the only successful treatment for CMTs is surgery, which consists of the removal of the altered glands and local lymph nodes [54]. In some cases of malignant CMT, chemotherapy and radiotherapy are administered. However, this is costly and there is limited information about the efficacy of the treatment [24]. The clinical need for biomarkers for early detection, treatment response and disease progression is therefore a research priority in this area [52].

3. Feline Mammary Adenocarcinoma (FMA)

3.1. What is FMA?

Feline mammary tumours predominantly present as adenocarcinomas [45]. Feline mammary adenocarcinoma (FMA) is the third most common tumour in cats, following lymphoid and cutaneous neoplasms [95, [113]]. The majority of FMAs are malignant [45]. and the the ratio of benign to malignant tumour presentations has been estimated to be between 4:1 and 9:1 [45]. FMA is a highly aggressive, hormone receptor negative cancer, with comparative pathology and poor prognosis similar to human TNBC. Domestic animals have a shorter overall lifespan and often show more rapid progression of cancer when compared to humans, thus allowing for treatment response comparisons to humans which is of interest not only to veterinary clinicians, medics and researchers but also to owners who often demand the use of the most advanced therapeutic tools for their pets [102]. However, interestingly, few studies have evaluated FMA's as a potential comparative cohort of patients to study human TNBC [114].

Mammary tumours in domestic cats are relatively common, occurring in approximately 31.8 and 20.4 in 10,000 intact cats and ovariohysterectomised cats respectively [7]. FMA typically occurs in older cats between the ages of 10 and 14 years, with the Siamese breed of cats having a higher risk even at a younger age [135]. In an early study of 179 malignant feline mammary tumours, Weijer, Head, Misdorp and Hampe [134], reported that the mean age at first detection, was 10.8 years with the average survival time following diagnosis being 12.3 months. Additionally, they reported that of the 179 studied, 120 presented with metastases, with most cancer progressing to the lymph nodes, lungs, pleura and liver. This finding of a predominance of malignancy in relation to mammary tumours in cats has been echoed by a number of studies and investigations [15]; [114]. There is abundant evidence to show that FMA shows an age associated, histopathology, biologic behaviour and a pattern of metastasis similar to human breast cancer [63] [134]. Hahn, Bravo and Avenell [113], have stated that naturally occurring tumours develop as frequently in animals as in man. Additionally, FMA also demonstrates a marked heterogeneity of molecular signatures between primary and metastatic lesions [127]. However FMA progresses at a much more rapid rate in our domestic pets when compared to human breast cancer. Reports of overexpression and amplification of cyclin A, and p53 nuclear accumulation, also indicate a similar molecular tumourigenesis [71].

3.2. FMA Histology and Prognostic/Predictive Indicators

In situ and invasive variants of FMA have been described. Prognostic parameters in FMAs have been characterised, including tumour size, histological grade, lymph node involvement and expression of proliferation markers [30] (Fig. 1)(Fig. 2). An example of a case of a histologically high-grade feline mammary adenocarcinoma from a 5 year old female neutered domestic short haired cat is shown in figure 1. A study by Burrai et al.,[17] examined tumours from 203 female cats of varying breeds, showing that the majority of FMAs were ER negative, PR negative and did not overexpress HER2 [17]. Cell proliferation as assessed by expression of Ki67 has been found to be higher in FMAs than benign tumours [30] Ki67 expression was also found to corresponds to the histological grade with higher grade tumours showing higher expression (Dias Pererira, Cavalheira and Gärtner, [30] concluded that prognosis of FMAs could potentially be assessed using the size of tumours, histological grading, lymph node involvement and expression of proliferative markers. Additionally, a study by Soares et al., [129] investigated the use of Ki67 as a prognostic indicator in

felines. 96 female felines with mammary carcinoma were examined. It was shown that a high Ki67 index was capable of predicting shorter OS, larger tumour size, more malignant tumours, presence of necrotic areas and low ER expression. In addition to its use as a prognostic biomarker, it was also proposed by Soares et al., that 14% Ki67 staining may be used as the optimal cut off for distinguishing high from low malignant potential and identifying high risk of disease progression. Despite these clear advances, further research is necessary to confirm the role of Ki-67 as a single marker of prognosis [30][114][129].Additionally, a number of histological grading systems commonly used in the human clinics have been applied to FMA, with the adapted Elston and Ellis scoring system [36] most commonly used with varying results. A relatively large study of 92 spontaneous invasive carcinomas of the mammary gland showed that histological grade using the Elston and Ellis grading system correlated with both overall survival and disease free survival [85]. Mills et al., [70] assessed the prognostic value of histological grading on 108 feline mammary tumours. They found Elston and Ellis grading to be a poor indicator of prognosis for feline patients and a poor indicator of overall survival. All studies agree that histologic grading of feline mammary carcinomas has the potential to significantly improve routine diagnostic evaluation and prognostication. Wiese et al., ([97]) investigated histological features including immunohistochemical evaluation of ER, PR, HER, CK5/6 and EGFR in order to investigate the growth pattern, tumour grade, mitotic index, the presence of necrosis, microcalcification, and a lymphocytic reaction associated with tumours in 24 cats with FMA. Fourteen out of 24 cats (58.3%) presented as triple negative, while 11 of those 14 (79%) were identified as "*basal-like*". They also showed FMAs, to be **(i)** aggressive tumours with a potential to metastasise and **(ii)** display morphological features similar to human breast cancer, suggesting that client owned cats may be a suitable spontaneous animal cohort for studying novel therapeutic approaches against human "*basal-like*" TNBC [97]. Finally, the study was supportive of the use of the Elston and Ellis grading system as a prognostic indicator; a grading system commonly used in human breast cancer [97]. An example of a case of feline 'basal -like' mammary adenocarcinoma showing positivity for CK5/6 from a 14 year old female neutered domestic short haired cat is shown in Figure 2 in comparison to a similar tumour in humans.

While proliferative markers, such as Ki67 and AgNOR, have been used as predictors of survival rates, the prognostic value of the expression of hormone receptors and HER2 is more controversial. Similar to human TNBC, the majority of feline tumours are ER negative [17], PR negative [44,17] and lack HER2 overexpression [124]. Millanta et al. [67] have shown that ER and PR expression cannot be considered definitively prognostic in cases of feline mammary neoplasia. The conflicting findings of most studies regarding the prognostic values of these receptors, is likely due to the low numbers of cases assessed [103]. As discussed previously, '*basal-like*' breast cancer is associated with immunohistochemical (IHC) positivity for the basal markers CK5/6, CK17, CK14 and EGFR, while 'triple negative' breast cancer is defined by negative expression of ER, PR and lack of overexpression of HER2 (Fig. 2). Wiese et al. [97] evaluated FMAs according to human TNBC IHC staining of ER, PR and HER2 as well as cytokeratin CK5/6 and EGFR and found that 79% of FMAs were negative for ER, PR and HER2 and exhibited a "*basal-like*" subtype. Soares et al., [127] used the St. Gallen International expert consensus panel to examine 61 felines with a diagnosis of mammary caner. Gene expression profiles were compared to local regional and distant metastasis. It was shown that triple negative "*basal-like*" mammary cancers showed lowest survival and shortest DFS. In addition, the majority of metastasis were triple negative with 50.7% of metastasis expressing CK5/6. A later study by Soares et al., published in 2016 further characterised the clinical and pathological features of feline mammary carcinomas and also supported the proposed heterogeneity of feline mammary carcinomas.[128] This research also eluded to the similarities between clinicopathological characterisation of both human and feline breast cancer. However importantly, Soares et al., commented on the difficulty faced when comparing clinicopathological results from felines to humans. Felines commonly develop multiple masses within the same mammary chain which can make interpretation of results more difficult. This is extremely rare in woman with "*basal-like*" breast cancers. As work in the interface between Veterinary Medicine and Clinical Medicine in humans progress, it is clear that spontaneous tumours in companion animals, offer the potential to study a very useful translational cohort [108].

3.3. Treatments: Surgery and Chemotherapy

Surgery remains the treatment of choice for feline mammary neoplasms, and may be used on its own or in combination with other modes of cancer therapy, such as chemotherapy [66]. In order to minimise the risk of recurrence, chain mastectomy is the preferred treatment plan, regardless of tumour size. A study by Gemignani et al., [110] examined 107 client owned cats, who underwent surgery for the treatment of a feline mammary neoplasm. Their study examined the incidence of disease progression and disease specific death identifying significant risk factors for disease progression including unilateral mastectomy, tumour ulceration and lymph node metastasis. Significant risk factors for disease specific death, included lymph node metastasis and development of distant or regional metastasis. Finally, their study also showed that chemotherapy treatment alone, was also associated with a significantly decreased risk of disease-specific death [110]. While results from this study support bilateral mastectomy and chemotherapy to improve progression free survival time, a study by Campos et al., [20] showed little or no increase in overall survival (OS) of patients who received chemotherapy together with surgery, compared to surgery alone [20]. Like humans, early intervention and treatment is extremely important for FMAs, with few effective therapies available for advanced disease.

Chemotherapy, although not the most adopted treatment for FMA, follows similar pathways to human TNBC. Doxorubicin is available as a single agent or in combination with cyclophosphamide [65,[128,129]. Similarly, carboplatin may be administered as a single agent or in combination with doxorubicin [20]. Despite the advanced treatment regimen, additional clinical trials are required to assess effective chemotherapeutic doses and combinations. A study was carried out by Campos et al. [20], to compare OS periods of felines subjected to varying treatment protocols including surgery alone and surgery together with adjuvant chemotherapy. Despite the fact that carboplatin is indicated for the treatment of both canine and feline malignant mammary gland neoplasms [56], no significant statistical difference in OS was observed between domestic cats who received surgery alone, compared to patients who received carboplatin in addition to surgery. Similarly, a study by McNeill et al. [65] tested whether doxorubicin-based chemotherapy improved the outcome for feline mammary carcinomas when compared to surgery alone. Of the 73 cats included in the study, 37 received surgery alone, while 36 received surgery followed by chemotherapy. Outcomes demonstrated that there was no statistically significant difference in survival of the felines who were treated with adjuvant doxorubicin following surgery [65].In an attempt to improve clinical management of feline mammary carcinoma, a study by Ferreira et al., [107]investigated the potential of TOP2α as a biomarker. It was shown that TOP2α gene aberrations are capable of predicting response to anthracycline based chemotherapy and amplification of TOP2α was associated with a better outcome for the feline patient cohort [107]. These studies clearly show a similarity between human and feline responses to treatment regimens with poor OS outcomes for both, using these chemotherapies and also highlight how research in the comparative medicine area has the potential to improve the overall survival of felines and TNBC patients.

COX-2 has also been investigated in FMAs. As is the case with human breast cancer, overexpression of COX-2 correlates with the

Table 1
Characteristics of human TNBC, FMA in domestic cats and CMT in domestic dogs, illustrating similarities and differences between subspecies.

	Human	Domestic Cat	Domestic Dog
Incidence	TNBC accounts for 10–15% of breast cancer diagnoses	"*Basal-like*" breast cancer accounts for approximately 79% of all breast cancer diagnoses	"*Basal-like*" breast cancer accounts for approximately 16% of all breast cancer diagnoses
	Humans are genetically diverse	Felines are genetically diverse	Canines are genetically diverse
Classification	Large proportion of TNBC are considered "*basal-like*"	Large proportion of FMA are considered "*basal-like*"	Small proportion of CMTs are considered "*basal-like*"
	"*Basal-like*" expresses CK5/6, Ki-67 and EGFR	"*Basal-like*" expresses CK5/6, Ki-67 and EGFR	"*Basal-like*" expresses CK5, p63 and P-cadherin
	Elston and Ellis grading system commonly used	Modified Elston and Ellis grading system commonly used	No optimised grading system available
Metastasis	Highly metastatic	Highly metastatic	Highly metastatic
	Metastasis to lung, liver and brain	Metastasis to lung, liver and brain	Metastasis to lungs and lymph nodes
Treatment	Respond slowly to treatments	Compressed Pathology; respond quickly to treatments	Compressed Pathology; respond quickly to treatments
	Standard care is combination chemotherapy in the adjuvant or neoadjuvant setting	Standard care is surgery, chemotherapies have been used	Standard care is surgery, chemotherapies have been use
	Clinical trials underway to investigate effects of COTI-2 on mutant p53	Little daata avaliuable n LiLittle data available on clinical trials for the treatment of FMA	Little data available on clinical trials for the treatment of CMT

([2], [15], [14], [19], [24], [35], [36], [52], [54], [55], [49], [59], [61], [63], [66], [91], [97], [101]).

aggressive and invasive potential of tumour cells, playing a role in carcinogenesis. In agreement with studies carried out on human breast tissues, a study by Millanta et al. [68] showed that 96% of FMAs expressed COX-2, in comparison to the normal mammary tissue, which demonstrated the absence of COX-2 expression. Studies investigating the use of COX-2 inhibitors have had limited success. A study by Borrego et al. [14] evaluated the efficacy of COX-2 inhibition using meloxicam; a nonsteroidal anti-inflammatory drug (NSAID) which inhibits COX-2, in combination with chemotherapy and surgery. The study involving 23 cats, showed no increase in their survival when treated with this NSAID and a chemotherapeutic combination. However, the dosage, dosing schedule and toxicity for meloxicam use, are not yet known, and it is possible that the dose used in this study was inadequate to inhibit COX-2 to a significant therapeutic level.

3.4. Clinical Trials and the Unmet Clinical Need

As discussed previously, spontaneously occurring FMAs affect a substantial number of cats globally each year [95]. Similar to humans, the aggressive phenotype and the propensity to metastasise, makes these tumours particularly difficult to treat resulting in a poor prognosis.Longstanding evidence exists to suggest that the lack of ER expression in cats may be suitable for evaluating therapeutic regimens for breast cancers that are not amenable to hormone targeting approaches [45]. It is also clear that, despite best efforts to treat felines using adjuvant chemotherapy in conjunction with surgery, OS remains consistently low. The finding of new more efficacious therapies is therefore a clinical priority and unmet clinical need. Despite extensive gene expression analysis in humans, there has been little analysis carried out on felines in this area. When cancer related genes; TP53, CCND1, FUS, YBX1, PTB1, c-myc and PXM2 were initially investigated there was little known about their function in feline mammary carcinomas [106]. Upon gene expression analysis of 27 mammary malignant tumours taken from female cats, it was shown that cancer related genes are associated but may also influence other genes directly. These genes show a complex cancer network which may in the future provide new molecular biomarkers [106]. Recent studies have also begun to investigate the roles of cytokines in feline breast cancer, in particular CXCR4 and its ligand CXCL12 [118]. Despite being extensively studied in human breast cancer, the importance of these cytokines was unknown in feline breast cancer. Analysis of mammary tumours from 115 felines showed that a complex interaction within the CXCR4/CXCL12 axis plays a substantial role in metastasis of breast cancer in cats. Therefore, Marques et al., also pointed to the potential importance of these cytokines in targeted therapy for the treatment of mammary cancer. Although promising, more specific work is needed to distinguish the exact relevance of CXCR4 and CXCL12 in each clinicopathological subtype of feline mammary cancer to predict novel therapeutic strategies in felines.

Immune profiling has provided revolutionary breakthroughs in the human cancer arena, however little investigations have taken place in veterinary patients. A study by Urbano et al., [133] investigated the levels of CTLA-4, TNFα, IL-6 in the serum of 57 female cats with a diagnosis of mammary cancer. It was found that increased CTLA-4 was associated with a "normal" or non "basal-like" status. Additionally, increased levels was also an indicator of smaller tumour size and overall better prognosis. The levels of CTLA-4 was attributed to the crosstalk between the immune cells and tumour microenvironment. Serum CTLA-4 levels were also positively correlated with serum TNFα and IL-6 levels [133]. Along the same line, a recent work by Nascimento et al., [122] has investigated the roles of serum programmed cell death protein-1 (PD-1) and its ligand programmed cell death ligand-1 (PD-L1). It was shown that levels of these proteins are significantly higher in cats with a diagnosis of "*basal-like*" mammary cancer. Recently, a PD-L1 inhibitor which promotes immune suppression has been approved by the FDA for use in human breast cancer, suggesting the potential for development of monoclonal antibodies as a therapeutic strategy for the treatment of "*basal-like*" breast cancer in feline patients also[122]. Additional work is clearly needed to establish the true effect of the immune landscape on feline mammary cancers, their development as novel biomarkers and to establish the potential role of novel immunotherapies in these patients.

While clinical trials are somewhat limited for cats, studies investigating the potential anti-cancer effects of virotherapy have been reported. Novel investigations into the MG1 strain of the *Maraba vericulovirus,* have shown potential as an anti-cancer vaccine in FMCs. Following preclinical research, five pet cats were enrolled to investigate the safety of the vaccine as a therapeutic [49]. Translational studies in cats suggested the tolerability and non-pathogenicity of the therapy, therefore displaying their potential use as a therapy for feline patients.

3.5. Canine BC in Comparison to Feline BC

CMT represent the most common neoplasm of intact adult female dogs with approximately 50% of these tumours being classified as malignant [115,121,126]. Certain subtypes have been reported to be highly metastatic such as anaplastic carcinomas, solid carcinomas and comedocarcinomas [124]. Few studies have classified CMTs based on the molecular characterisations of human breast cancers. However, most of these studies indicate the occurrence of 'triple negative' and *basal-like*' subtypes which are associated with poor prognosis [38,115]. In one of these studies carried out by Gama et al.,[38] malignant CMTs

were characterised based on the phenotypic classification of human breast cancer, using a panel of six markers (ER, PR, HER2, CK5, p63 and P-cadherin), which allowed for the identification of four molecular phenotypes; luminal A, luminal B, HER2-overexpressed, and "*basal-like*". The "*basal-like*" sub-phenotype was characterised by a high grade and high proliferation rate, as well as positive expression for cytokeratin 5 (CK5), p63 and P-cadherin. This subtype was also associated with a shorter disease free survival time and overall survival. However, the incidence of *"basal-like"* metastatic disease in canines was shown to be relatively low. In a study by Kim et al., [115] they examined 241 canines diagnosed with canine mammary carcinoma. Of these 241 tumours, 45 cases (18.7%) were considered triple negative with 34 out of the 45 considered "*basal-like*". Based on this studies, one might suggest that "*triple negative"* or *"basal-like*" breast cancer accounts for a very small proportion of all canine breast cancers (**Table 1**). It is however important to bear in mind that the incidence of metastasis and prognosis is very difficult to predict in the dog, as there are very few large scale studies of CMT [115].

Histological grading of CMT has also been shown to be a prognostic indicator. Several grading systems are used with modifications of the numeric method of Ellston and Ellis being the most common [111,132]. Zapulli et al., [102] reported that age-adjusted overall cancer incidence per 100,000 individuals per year is comparable in humans and domestic animals with 300 in humans, 381 in dogs and 264 in cats, however based on canine studies and feline studies discussed in this review, it is clear that the domestic cat represents a more superior opportunity to study human breast cancer, in particular TNBC (Table 1). Another study in support of this statement, was carried out by Caliari et al., [19] Specifically, they examined tumours from 81 cats with FMA. Of the 81 cats examined, 93% were malignant tumour presentations. When this same ratio is calculated in canine patients, it is clear, that a much larger proportion of cats develop malignant breast cancers compared to canines. In addition, Caliari et al., [19] reported that the majority of malignant feline tumours appear phenotypically similar to TNBCs. This contrasts with studies of malignant canine tumours, where a very small percentage (24%) display the *"basal-like"* phenotype. To summarise, as seen in Table 1, it has been found that the majority of malignant feline mammary carcinomas display a similar phenotype to human TNBC, therefore potentially representing an available comparative cohort to study both human and feline TNBC. Comparative studies of clinical and pathological features of TNBC and FMA would increase our knowledge of cancer pathogenesis and may result in the finding of novel targeted therapies in both felines and humans [102,113,114,135].

Despite the clear benefits of comparative studies, substantial challenges also exist, particularly in using the cat as a comparative model. Clinical trials involving the domestic cat are extremely scarce in the literature [130]. However, it is unclear whether this is due to lack of resources or lack of knowledge of the population at risk. While LeBlanc, Mazeka and Khanna [116] proposed that the value of comparative oncology lies in the critical drug development questions that cannot be answered by human trials, or conventional preclinical modes, it has also been noted that the field has mainly focused on tumour bearing dogs. This may represent the majority of what presents to vets or may also be due to the fact that the understanding of genetic changes and mechanisms resulting in canine cancers are much greater than in cats [130]. For example, the complete feline genome became available in 2007 [123], while the canine genome had been available since 2005 [131].

It is also impossible to ignore the fact that larger numbers of humans with breast cancer decide to take part in scientific research. Incorporation of human breast cancer patients into scientific studies typically include a large proportion of the breast cancer patients that have been diagnosed. This is not the case for the feline cohort, as the selection criteria are often subjective, and the population at risk are not well known [130]. When subjective criteria are used, all of the cases of FMA in the population are not diagnosed. This issue may induce selection and diagnostic biases in cats with mammary cancers. Additional challenges such as a lack of funding for comparative research, is also a substantial challenge facing researchers as human cancer research organisations tend to be incognizant to comparative oncology [130]. There can also be challenges with enrolment and engagement with pet owners. When cat owners were asked whether they would consider enrolling their pet cat in a clinical trial, many were found to be unsure [112]. This however represents opportunities at the educational level to improve recruitment and awareness of the importance of comparative oncology.

4. Extracellular Vesicles (EVs) and Exosomes

4.1. What are Exosomes?

Extracellular vesicles (EVs), including exosomes, microvesicles, oncosomes and microparticles, are nanoparticles found in all biological fluids examined to date [57]. EVs are typically composed of a lipid bilayer formed from the plasma membrane, which encases genetic material, proteins and lipids [57]. First described over thirty years ago [51], these EVs are fundamental in cell-cell communication and the transmission of disease states [34]. Since its discovery, the term *exosome* has been used loosely to describe an array of EVs. However, exosomes are best described as "*extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body, with the plasma membrane*" [34]. Typically between 50 and 150 nm in size, exosomes characteristically display CD63, CD9 and CD81 tetraspanin proteins [100]. Exosomes may also carry major histocompatibility complex (MHC) class II and heat shock proteins as well as ALIX and TSG101 [5].

In addition to intracellular communication, recent studies have demonstrated that exosomes may play an important role as vectors for drug delivery. An early study by Raposo et al. [82], showed that exosomes are capable of carrying the MHC-peptide complexes that can be recognised by T-lymphocytes to promote antitumor immune responses *in vivo*. Exosomes are highly representative of their cells of origin, as they contain DNA, RNA, proteins, lipids and other molecular constituents of the cell from which they have been released. Exosomes have also been associated with propagating anti-cancer drug resistance and the development of metastasis [64]. Upon the discovery of exosomes, their role in science was largely ignored as they were deemed "*waste containing vehicles*" or "*a means of cellular disposal*". However recent analyses have reported a ten-fold increase in publications involving exosomes [34], identifying their fundamental importance in normal physiology and pathophysiology [41].

4.2. The role of exosomes in TNBC Metastasis and Disease Progression

Incremental exosome research has shown that exosomes are also implicated in the pathogenesis of disease, including TNBC. Extracellular vesicles are key players of intercellular communication. The more active release of exosomes by cancer cells has been acknowledged as a general cancer trait [89]. However, it is not clear whether exosomes may be playing a role in TNBC metastasis [27,89]. Research by O'Brien et al. [72], investigated the capabilities of exosomes to transfer phenotypic traits that are representative of their cells of origin to recipient cells, potentially contributing to the development of distant metastasis. The results showed that in TNBC, communication *via* exosomes may confer aggressive behaviour to secondary recipient cells. It was also noted by O'Brien et al. [72] that future studies focused on identifying the specific molecules involved in proliferation (by profiling the contents of these exosomes) and including analyses of larger cohorts of serum specimens was warranted to expand our understanding of the functional relevance of exosomes in TNBC. A further study by Ozawa et al. [74] investigated the role and mechanisms of EVs derived from

TNBC cells in modulating proliferation and cytotoxicity to non-tumourigenic breast cells. Their study showed that EVs isolated from TNBC cells were indeed capable of inducing proliferation in non-tumourigenic breast cells. This functional change seen in recipient cells may be due to changes in genes and miRNAs expression causing them to proliferate, under apoptosis, invasion other tissues and migrate to other sites [74].

Despite the clear lack of research and investigation of exosomes in domestic cats, recent studies have shown that exosomes can be successfully isolated from the serum of dogs, cats and horses [119]. Similar to humans, when exosomes of veterinary patients were profiled in order to detect whole transferrin receptor in an effort to detect iron deficiency, heterogeneity of relevant markers, soluable transferrin receptor and cytoplasmic domain was reported between individuals of the same species [119]. This shows that it is possible to profile exosomes from the serum of veterinary patients. It was previously proven by Lawson et al. [57], that extracellular vesicles could be detected in bodily fluids from mammalian and non-mammalian laboratory species, using non-staining techniques such as nanoparticle tracking analyses (NTA) and flow cytometry. They also showed that EVs from equine and feline plasma contain a lipid bilayer, as well as cargo such as genetic material including DNA and RNA, proteins and lipids [57]. Their study concluded that exosome research is providing new insights into how cells communicate within different species, showing great potential as biomarkers for different diseases including cancers in both human and veterinary patients. Understanding more about the role of EVs in feline diseases including cancer, may enable the development of a new generation of diagnostic and prognostic tools for use in the veterinary clinic. Lawson et al. [57] stated that "*development of assays to measure EVs from veterinary species will enable high quality care and enhanced welfare of patients*".

However, some research has been carried out in canines that suggests that the profiling of EVs from domestic cats may yield some interesting results. Fish et al., [109] showed that breast cancers from cell lines shed exosomes that contain microRNAs into the tumour microenvironment and circulation, therefore potentially representing biomarkers of metastasis and tumour phenotype. When exosomes from culture media from CMT derived cell lines were compared to exosomes from non-cancerous cells, it was found that certain microRNAs are upregulated in the exosomes secreted from tumour cells. These microRNAs are predicted to target biologically relevant hormone receptors and oncogenic pathways [109]. This extremely revolutionary study in the area of exosomes and veterinary medicine may only represent the tip of the iceberg for these nanoparticles. Therefore, in relation to TNBC and FMA, a comparative approach to exosomal profiling may provide the key to unravelling the molecular complexity and aggressiveness of TNBC which may lead to changes or improved treatments for both feline and human populations.

4.3. Exosomes and Chemoresistance

Chemoresistance refers to the resilience of the cancer cells to avoid apoptosis-induced death by a chemotherapeutic agent. The widely accepted mechanism of chemoresistance involves changes in the tumour microenvironment, which is hypothesised to depend heavily on exosomes. MicroRNAs (miRNAs) are known to play a role in the formation of cancer stem cells and subsequent Epithelial-to-Mesenchymal Transition (EMT), forming hallmarks of breast cancer [83]. Interestingly, there is increasing evidence that EVs might have a role in chemoresistance. In 2018, a study looked at the significance of miRNA-155 in mediating chemoresistance in breast cancer cells, with the potential for the development of miRNA-155 as a therapeutic target. The study showed that, exosomes are capable of binding to neighbouring cells and modifying recipient cell phenotypes on uptake; the miRNA carried by exosomes is translated into recipient cells, potentially mediating these changes. Upon further analysis of the role of the microRNA-155 in chemoresistance, it was shown that cells that received exosomes that had high levels of miR-155, were significantly more resistant to chemotherapeutic agents such as doxorubicin and paclitaxel [83]. A role of exosomes in TNBC chemoresistance has been confirmed by Ozawa et al. [74], where studies showed that extracellular vesicles from TNBC cells are capable of inducing chemoresistance in normal non-cancerous breast epithelial cells.

Although the role of exosomes in mediating chemoresistance in feline patients has not been investigated in as much detail, exosome derived microRNAs have been shown to be significantly associated with the progression of renal dysfunction in felines [50], demonstrating the potential role of exosomes in the pathogenesis of disease. Similarly, the role of miRNAs in cardiomyopathy has recently been investigated [99]. The parallels between human TNBC and FMA support investigation of the potential of EVs as biomarkers, and mediators of chemoresistance in veterinary medicine.

5. The Future of Comparative Oncology

5.1. Liquid Biopsy for Diagnosis

A *liquid biopsy*, also known as a *fluid biopsy,* refers to the sampling of non-solid biological tissue, mainly blood in the form of plasma or serum to inform on some disease state. Altho Although a routine clinical procedure,the use of these liquid biopsies for the isolation of exosomes or other particles harbouring pathophysiological information is novel. Exosomes are cellular reflections of their cells of origin. Therefore, the use of liquid biopsies in this way has been described as a revolutionary technique harbouring a wealth of undiscovered genetic information. A study by Stover et al. [90], demonstrated the potential impact of the liquid biopsy for humans in the cancer diagnostic setting. Their study showed that metastatic TNBC could be characterised based on the Cell-Free DNA (cfDNA), harvested from the blood samples of 164 patients whereg genomic characterisation of the metastatic TNBC was carried out exclusively on the cfDNA alone. It was demonstrated that information regarding metastasis and chemoresistance could be obtained using this liquid biopsy approach and further demonstrated a novel aspect of breast cancer profiling as well as the power of the liquid biopsy in the clinical setting [90].Similar to humans, liquid biopsy samples from domestic cats also present the opportunity to characterise cfDNA of feline mammary adenocarcinoma. A study by Leal et al., [117]used cfDNA to detect residual disease in felines with gastric cancer. They were able to identify patients who had benefitted from perioperative gastric cancer treatment [117]. It can therefore be deduced that a similar approach to monitoring disease progression in feline mammary adenocarcinoma has huge potential in the clinical setting. As with humans, exosomes isolated from the blood of felines with mammary cancer has the potential to inform on disease progression and prognosis. EVs have been isolated, identified and characterised from felines with a diagnosis of mammary cancer [125]. It was concluded that the preliminary study which identified and characterised EVs from felines with mammary tumours represents a new interesting field with several applications and limitless potential [125]. Harvested exosomes therefore have the potential to inform on the location of metastasis and resistance to chemotherapy in the clinical setting in both human and veterinary medicine.

5.2. Conclusion: A Comparative Oncology Approach

The poor prognosis, lack of specialised or effective treatment regimens and susceptibility to the development of metastasis of TNBC is the epitome of an unmet clinical need. Like TNBC, FMAs are similar to human "*basal-like*" breast cancer (a subset of TNBC). They too carry a poor prognosis, and also lack efficacious treatments. Felines typically display a compressed pathology compared to humans. Therefore, changes in their disease, such as in response to new treatments may be

seen more quickly thus supporting the comparative oncology approach. Comparative oncology may reveal novel information that could not be inferred from rodent models, and cats with FMA can contribute to and benefit from TNBC research. As such, this is in keeping with the definition of comparative oncology; to benefit both humans and animals equally.

A wealth of exosome research has occurred in recent years, which has been particularly interesting for TNBC researchers and patients alike. The potential roles of exosomes in TNBC metastasis and chemoresistance warrants further investigation which may unlock their true potential as biomarkers. In a collaborative environment, exosomes may be harvested from liquid biopsies of both humans and felines to potentially unravel the complex characteristics of both triple negative breast cancer and feline mammary adenocarcinoma to the potential greater good and care of both.

Declaration of Competing Interest

There are no conflicts of interest to disclose.

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