Review

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The Role of Canine Models of Human Cancer: Overcoming Drug Resistance through a Transdisciplinary Approach "One Health and One Medicine"

Sara Gargiulo [1,2,*], Lidovina Vecchiarelli [3], Eleonora Pagni [3,4] and Matteo Gramanzini [6]

[1] Institute of Clinical Physiology, National Research Council, Via Fiorentina 1, 53100 Siena, Italy
[2] Core Research Laboratory (CRL), Istituto per lo Studio la Prevenzione e la Rete Oncologica (ISPRO), 53100 Siena, Italy
[3] Animal Welfare at Animal and Plant Health Agency, Department for Environment Food and Rural Affairs, Midlands, UK
[4] Veterinary Medical and Oncology Centre "Etruria", Via Sandro Pertini 5-6, 53035 Monteriggioni, Siena,
[5] FSA (Fondazione Salute Animale), Via Trecchi 20, 26100 Cremona, Italy
[6] Institute of Crystallography, National Research Council, Strada Provinciale 35d, n. 9-00010, Montelibretti, 34149 Rome, Italy
* Correspondence: sara.gargiulo@cnr.it

Simple Summary: Resistance to oncotherapy represents a significant challenge for both human and canine patients. Comparative oncology has highlighted remarkable similarities between canine and human tumours with respect to genetic and tissue composition factors involved in drug resistance. Spontaneous canine cancer models more accurately recapitulate the oncogenesis and complex, heterogeneous microenvironment of human tumours than laboratory animals in which neoplasia is induced do. Companion dogs share numerous exposure factors with humans and show clear similarities in molecular and histological features, as well as in immune and treatment responses. An appropriate model is essential for successfully implementing precision medicine strategies. This comprehensive review aims to highlight the critical role of canine models of human cancers in advancing current knowledge of drug resistance mechanisms, helping to improve the development of treatments that address this relevant issue through a "One Health, One medicine" translational research approach.

Abstract: Introduction: Chemotherapy is a primary treatment option in human and veterinary oncology. Like humans, canine patients often develop drug resistance. Comparative oncology is gaining increasing interest, and spontaneous tumours of companion dogs have emerged as a powerful resource for better understanding human cancer. The genetic, molecular, and histological features of tumours in dogs are more closely related to those in humans than the ones in laboratory animals, including complex mechanisms of drug resistance.**Methods:** A comprehensive literature search was conducted in the electronic database Clarivate Web of Science (WOS): Medical Literature Analysis and Retrieval System Online (MEDLINE) from 1990 to 2025 (updated 2025-01-20). The final set includes 59 relevant full-text English articles. **Results:** Literature findings suggest that canine spontaneous tumours are valuable model systems with important translational implications for identifying novel mechanisms of chemotherapy resistance shared with humans and may help advance the current standard of care in precision medicine. **Conclusions:** We have provided an updated overview of the role of canine tumour models to study oncotherapy resistance, focusing on limitations and opportunities for advancement. Despite complementary benefits of such models in translational oncology research, their relevance remains underestimated. Strengthening the collaboration between the human and veterinary medicine professionals and comparative medicine researchers, and getting the support of interdisciplinary institutions, could contribute to addressing the problem of multidrug resistance for both human and canine patients. Future research may promote using canine spontaneous tumours as promising translational therapeutic models for human chemoresistance, through a multidisciplinary approach based on the emerging "One Health, One Medicine" paradigm.

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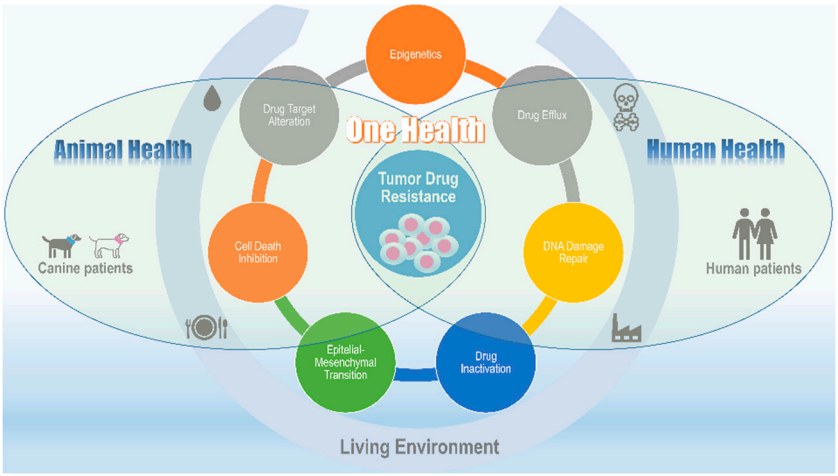

Keywords: drug resistance; comparative oncology; canine model; spontaneous tumour systems; One health; One medicine

1. Introduction

The identification of mechanisms underlying primary and secondary resistance to therapies in oncology is an active field of research.

Additionally, the need to promote an interdisciplinary approach in oncology and to improve the choice of valid experimental preclinical models to address the problem of drug resistance (DR) in cancer treatment is widely recognized [1].

Nowadays, canine cancer cell lines and spontaneous canine tumours have been recognized as valuable models for studying cancer biology, and testing novel therapeutic approaches in vitro and in vivo that could benefit both species. However, efforts to integrate collaboration between human and veterinary medicine, both in clinical and research settings, are still under development, hindering the possibility of knowledge sharing and optimizing the expertise and data potentially available to develop more effective strategies to defeat DR [2].

Companion dogs can be affected by many of the same types of cancer that occur in humans, including breast, prostate, and lung cancers, glioma, melanoma, and lymphoma/leukaemia; comparative oncology studies have highlighted extensive histological, genetic, and molecular similarities between the two species [3,4]. Furthermore, comparative cancer epidemiology through Animal Cancer Registries has been recognized as a precious source of complementary information for human oncological prevention [5]. However, the role of animal models of human cancer in clinical therapeutic research is still a matter of debate. Overall, tumour biology appears to be more closely related among spontaneous tumours occurring in both dogs and humans than those are induced in immunocompromised laboratory rodents by chemicals, human tumor cell transplantation, or genetic engineering techniques [3,6]. Dogs are exposed to similar environmental risk factors to humans compared to the controlled artificial parameters of a laboratory animal facility. Canine models may also better reflect the complexity of the tumour microenvironment and its interaction with host immunity influencing the therapeutic response [7]. Furthermore, breed-specific predisposition for certain types of tumours allows us to investigate the role of genetics in tumor development [8]. Also, the shorter life expectancy of dogs and their faster rate of cancer progression compared to humans reduce the length of clinical studies and the time needed to extrapolate useful results on DR to humans, at lower costs and with less stringent regulatory requirements [9,10]. Considering the current prevalence and social role of dogs in Western world families, and the modern ethical attitude aimed at protecting their well-being and health, sample collection, surgical interventions and imaging are currently widely implemented in dogs with cancer. Indeed, clinical veterinary medicine has reached a high level of specialization, applying diagnostic and therapeutic standards in veterinary medical centers comparable to human medicine. In general, the rate of effective translation of therapies from studies on rodent models to clinical trials is very low [11]. Interestingly, dogs are often treated with the same chemotherapeutic drugs used in human patients and, after initial success,

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develop similar DR. Therefore, clinical investigations on companion dogs could be well accepted by the public opinion, since they will bring benefits beyond humans to veterinary oncology.

The attractiveness of the canine comparative models in the fight against cancer of humans has emerged since 2003, when the National Cancer Institute's Center for Cancer Research (CCR) started the Comparative Oncology Program (NCI-COP; ccr.cancer.gov/resources/cop/) [11]. Preclinical studies in dogs with cancer have helped to test innovative approaches of immuno- and gene therapy, as well as optimized drug delivery tools before human clinical trials [7]. Furthermore, canine cancer cell lines, both continuous and derived from primary tumours, have been advantageously used as an in vitro model to study the molecular pathways involved in tumour development and resistance to chemotherapeutics.

DR is a relevant clinical challenge limiting the success of chemotherapy in both human oncology and veterinary medicine, and the cellular mechanisms of DR in dogs appear to overlap with those in humans due to the evolutionarily conserved genetic pathways involved [12,13]**.** Despite the emerging promotion of the One Medicine paradigm and One Health education, these powerful animal models remain vastly underutilized to study cancer biology and DR, likely due to limited opportunities for interdisciplinary convergence between clinical and research institutions. Indeed, identifying and targeting DR tumour cells appears to be a rational approach to improve current standards of care, and the dog may represent a valuable large animal model to study this research challenge, thereby facilitating the use of approaches similarly to humans. Canine models could contribute to gain new insights into the development of anticancer drugs that promote the reversal or prevention of DR; their complementary use with other available experimental models would ensure more comprehensive results. Clearly, dogs and humans may show species-specific differences in the pharmacokinetic and pharmacodynamic profiles of drugs that could potentially affect their efficacy and safety [14]. An overview of current knowledge on chemotherapy resistance is beyond the scope of this review. Our aim is to explore the contribution of canine models in the study of tumour resistance to chemotherapy, the capabilities and shortcomings of research efforts, and possible strategies to effectively integrate available resources to address this specific public health challenge with a high social impact.

From this perspective, a constructive scientific debate could be stimulated among researchers and clinicians on the opportunity to implement an integrated use of animal models to provide new insights into overcoming resistance to chemotherapy drugs.

2. Materials and Methods

2.1. Search Strategy

The search strategy was designed to capture all relevant paper investigating the use of spontaneous canine tumours as models to study DR issue. The research project was prospectively (pre)registered in Open Science Framework (OSF) on 05th, February 2025 (https://osf.io/h8veq/?view_only=d3b2241cacc64c87afa615cd1fa2ff6f; DOI 10.17605/OSF.IO/H8VEQ). Clarivate Web of Science (WOS): Medical Literature Analysis and Retrieval System Online (MEDLINE) electronic database was searched and screened in 22 January 2025 using the following string of search terms: TS=((canine model) AND (human) AND (neoplasia OR tumour) AND (oncotherapy OR chemotherapeutic OR drug) AND (resistance)) to identify pertinent article from 1990 to 2025 (updated 2025-01-20). The final set includes 89 full-text English articles, which were exported into a single Reference Manager file (*supplementary material, RM file*).

2.2. Selection Criteria

For inclusion in this study, the following were considered eligible: peer-reviewed case report, comparative studies, classical articles, journal articles, journal reviews, randomized controlled trials, Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, P.H.S, Research Support, N.I.H., Extramural, and technical report that focused on spontaneous canine tumours models for the overall DR study. Titles, abstracts and full texts were critically examined for relevance and the following articles were excluded:

referring to resistance to therapies other than anticancer drugs (e.g. antimicrobials, antiparasitics) and clearly outside the scope of the review;

- or where DR was not the primary focus of investigation (e.g. compound toxicity, targeting strategies, or drug delivery);

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- or where the in vitro/in vivo canine model does not have an evident relevance in the context of the study;
- or in which animals other than the canine model were used (i.e. laboratory mice or other companion animals) and clearly outside the scope of the review;
- or reviews that summarize the results of relevant original articles already included in the search list;
- or in which the preliminary data are provided by the same authors of a main work;

Ultimately, 59 articles were identified as appropriate for summarize the findings of the present study, as presented in Figure 1.

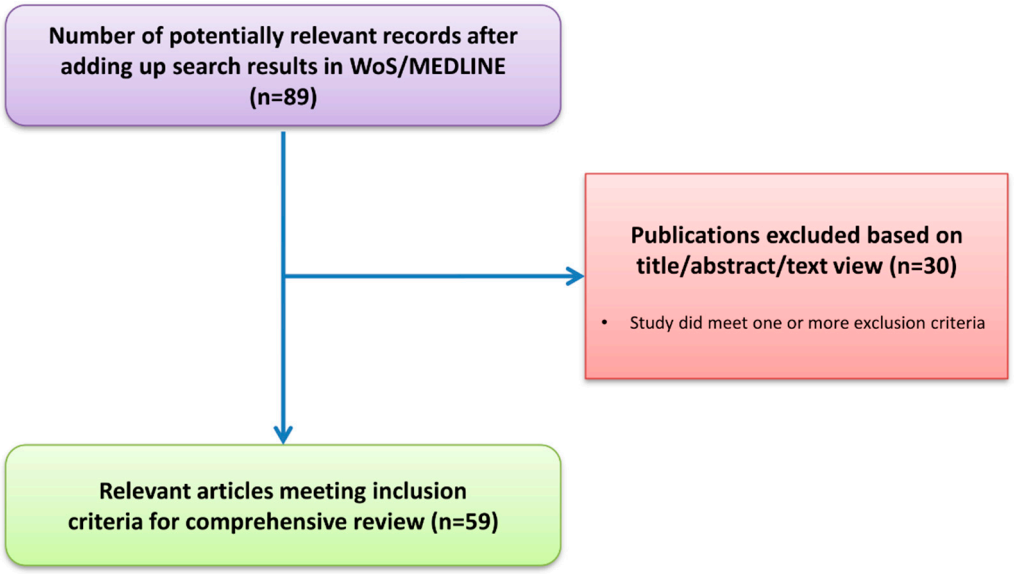

Figure 1. Flow diagram of the manuscript selection process.

3. Results

In this review, we discuss the literature findings that highlight the value of canine models in comparative oncology, based on the main mechanisms of DR shared with humans. Although we have selectively discussed the spectrum of the most relevant DR biological pathways displayed by tumour cells, the relationship between them is obviously more complex.

A comparative summary of DR mechanisms shared by human and canine tumours is shown in Figure 2.

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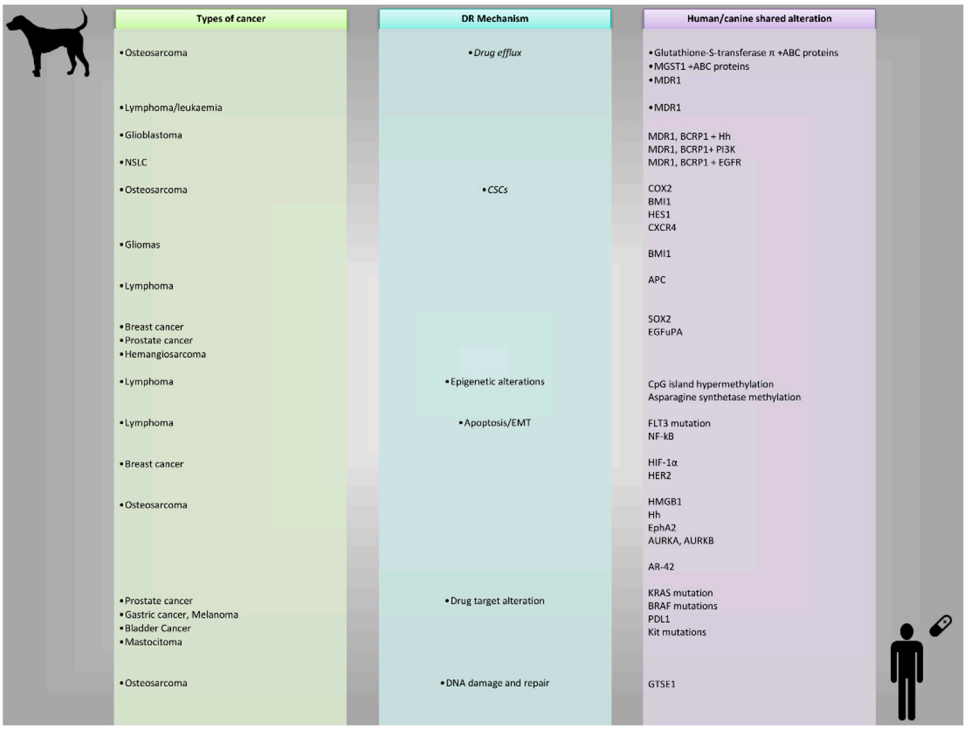

Figure 2. Schematic diagram outlining comparative oncology in companion dogs and humans.

3.1. Efflux of Chemotherapeutic Agents

Enhanced drug efflux by the ATP-binding cassette (ABC) superfamily of transmembrane transport proteins is one of the most frequent mechanism of DR. Their expression is often elevated in chemotherapy-resistant tumours, both in humans and dogs, resulting in active efflux of chemotherapeutic agents. In particular, overexpression of P-glycoprotein (P-gp) also known as multidrug resistance 1 (MDR1), multidrug resistance-associated protein 1 (MRP1), multidrug resistance-associated protein 2 (MRP2), and Breast Cancer Resistance Protein (BCRP) has been linked to multidrug resistance (MDR) and poor prognosis in both human and canine patients by hindering the intratumoral uptake of chemotherapeutics. Interestingly, ABC transporters are constitutively expressed by the blood-brain barrier (BBB), a further challenge for effective pharmacotherapy against brain tumours. Based on this evidence, the combined use of conventional drugs and ABC transporter inhibitors has been proposed to manage MDR.

Among the first animal models that highlighted the potential efficacy of P-gp blockers against MDR were Collie dogs that showed increased sensitivity to the neurotoxic effects of ivermectin, a widely used antiparasitic and P-gp substrate, which was related to impaired P-gp function in the BBB due to a mutation in the MDR1 gene. Consistent effects of ivermectin were shown in studies on a mouse model lacking P-gp function, which led to a compromised BBB and subsequent increased central nervous system (CNS) permeability to xenobiotics [15]. Overall, this dog breed represents a useful model of naturally occurring gene invalidation, which could be exploited to inform human therapies targeting MDR mechanisms.

Similarly, one of the first clinically relevant mechanisms of chemoresistance demonstrated was cisplatin resistance mediated by the enzyme glutathione-S-transferase π in a spontaneous model of canine osteosarcoma [16]. The validity of this spontaneous cancer model for human studies was already clearly recognized. In fact, it helped demonstrate that overexpression of glutathione-S-transferase π was correlated with limited accumulation of cisplatin in cells in vitro and reduced survival of canine patients, partly due to the action of an ATP-dependent pump that would actively clear drug molecules complexed to the enzyme from tumour cells [16]. Initial comparative studies have shown that the complementary DNA of the gene encoding P-gp, and consequently its fundamental function of active efflux of potentially toxic xenobiotics from cells, show a high similarity among vertebrates. Nevertheless, there are species-specific differences in the promoter regions that regulate gene expression. Indeed, canine P-gp showed comparable function to that of humans, but differences in the downstream gene promoter suggest that its expression may be regulated differently in canine tumour cells, which overall supports interest in further investigation

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of this translational issue [17]. One of the first studies using the canine osteosarcoma cell line OS2.4 demonstrated that doxorubicin treatment was able to select clones resistant even to vincristine through overexpression of P-gp, similar to the results obtained in human osteosarcoma cell lines and biopsy specimens [18]. These findings first suggested the utility of the canine osteosarcoma model to study MDR mechanisms in the human counterpart. A subsequent clinical study, which performed an expression profile analysis on genes regulating cell proliferation, metastasis, and DR in canine osteosarcoma patients, found that the MGST1 and HMGB1 genes were overexpressed in dogs with MDR as in humans with other tumours. Overall, MGST1 has been shown to be involved in the detoxification of cells from various cytotoxic drugs, while HMGB1 mainly regulates DNA damage repair and cell cycle, with antiapoptotic effects [19]. These in vivo results were consistent with in vitro studies conducted using canine lymphoma and leukaemia cell lines. The canine B-cell lymphoma cell line GL-1, exposed to doxorubicin, developed a P-gp-mediated resistance even to vincristine, which was reversed by verapamil, while it remained sensitive to cisplatin [20]. Doxorubicin and vincristine are known to be involved in P-gp-related human MDR, which interferes with the accumulation of these drugs within cells. P-gp function was inhibited by masitinib, a tyrosine kinase inhibitor, increasing the uptake of the substrate rhodamine123 (Rh123) and reversing doxorubicin resistance in the canine lymphoid cell line GL-40, a doxorubicin- and vincristine-resistant subclone selected from GL-1 cell line after incubation with increasing concentrations of doxorubicin [21,22]. Indeed, the P-gp inhibitor PSC833 completely reversed resistance to chemotherapy and Rh123 uptake, while treatment with masitinib in the GL-1 cell line, which does not express P-gp, had no evident effects on doxorubicin-induced cytotoxicity [21,22]. Based on these results, the in vitro canine model, translationally comparable to human non-Hodgkin lymphoma, could represent a promising experimental system for screening P-gp-mediated DR.

Together with the molecular findings from the analysis of canine lymphoma samples [23], these results also suggest the opportunity to investigate the potential clinical utility of targeting P-gp to improve therapeutic efficacy in canine and human patients with MDR to cytostatic agents. However, for brain tumours such as glioblastoma, obstacles to achieving effective drug concentration at the tumour site are represented not only by P-gp expressed on brain endothelial cells, but also by the limited permeability of the BBB. The drug efflux pumps MDR1 and BCRP1 are expressed on the luminal side of brain capillary and, together with tight junctions between endothelial cells, form the BBB that limits the brain penetration of lipophilic drugs. The role of these efflux pumps on drug distribution in the CNS has been extensively evaluated using the experimental Madin-Darby canine renal epithelial cell line (MDCKII) system.

Interestingly, an initial experiment using MDCKII cells stably expressing human MRP1 and its variants in the N-terminal regions showed that this region, although highly evolutionarily conserved, would not be involved in the transport function of MRP1 [24]. Considering the crucial role of drug efflux transporters in chemotherapy resistance of human tumours, this in vitro canine model highlighted the need for detailed analyses to define the role of different protein domains and provided preliminary useful data for the future design of clinically relevant inhibitor molecules.

By using MDCKII cells overexpressing P-gp, MRP1 or MRP2, HhAntag691 was shown to be both a Hedgehog (Hh) pathway inhibitor and an ABC transporters inhibitor, and to be able to reverse colchicine resistance. These findings suggest the potential use of agents simultaneously inhibiting Hh signalling and ABC transporters as promising anticancer drugs against cancer stem cells (CSCs) and brain tumours [25].

Similarly, the potential development of inhibitors of polymerases (PARPs), which play a key role in modulating DNA repair, has been studied in the same cell model overexpressing MDR1 or BCRP1, with the aim of synergizing conventional cytotoxic drugs [26].

Interestingly, in vitro intracellular accumulation of rucaparib was significantly reduced in cells expressing both MDR1 and BCRP1 compared to control cells, while treatment with specific efflux pump inhibitors increased its uptake, demonstrating that this compound represents an efflux substrate for both pumps. Consistent with in vitro results, in vivo findings obtained using subcutaneous murine glioblastoma xenografts highlighted that rucaparib could potentially act synergistically with temozolomide, while the lack of the same effect in orthotopic glioblastoma mouse models confirmed that MDR1 and BCRP1 significantly limit the potential utility of PARPs inhibitors in clinical use [26]. Related to this issue, a study on MDCK cells transfected with human P-gp, human BCRP or mouse BCRP1 clearly indicated that the promising PI3K/mTOR inhibitor GDC-0084 was not

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a substrate of these ABC transporters [27]. These preliminary data suggested that the PI3K pathway, frequently altered in glioblastoma, could represent an interesting target for the treatment of brain tumours using agents that freely cross the BBB, laying the groundwork for the following preclinical studies and clinical trials. In a subsequent investigation, the MDCK-MDR1 cell line served as a useful in vitro model to test the ability of a novel iron oxide-based doxorubicin nanocarrier to cross the BBB and bypass the MDR. It effectively enhanced chemotherapy uptake in both this cell model and in U251 human glioblastoma cells, when combined with transient opening of BBB tight junctions [28]. With the common goal of improving drug delivery into the brain, the same glioblastoma cell line and MDCK cells transfected with BCRP (MDCK-BCRP) were appropriately used to study the inhibitory effect of elacridar on P-gp and BCRP at the BBB level [29]. Elacridar accumulation in transfected cell lines was significantly lower than in wild-type cells, suggesting that P-gp and BCRP limit its uptake. Consistent with in vitro findings, in vivo brain distribution of elacridar in mouse models was influenced by active efflux exerted by P-gp and BCRP, which interestingly appeared saturable. This study highlighted that high concentration of elacridar are necessary to saturate the transporters at the BBB, thus effectively improving the delivery of a chemotherapy drug into the brain and overcoming the DR in brain tumours [29].

Numerous other examples demonstrate the robustness of MDCK cells for translational studies of efflux transport in anticancer DR research.

MDCK-MDR1 and MDCK-BCRP cells supported preclinical and clinical evidence that osimertinib, a potent epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), may be a promising therapeutic option in the management of brain metastases from non–small cell lung cancer. Although in vitro canine models confirmed that osimertinib is a substrate for the efflux transporters P-gp and BCRP, which commonly limit chemotherapy delivery to the CNS leading to DR, it showed greater BBB penetration than conventional agents in murine models and patients [30,31]. These findings suggest interesting avenues for improving the efficacy of treatment against brain lesions in patients with acquired resistance to current EGFR-TKIs, and highlight the utility of in vitro canine models for screening transporters in the development of new anticancer drugs. In the perspective of efforts to discover new P-gp inhibitors that counteract MDR, MDCK-MDR1 cells have been useful to test the P-gp inhibitor HZ08, highlighting its ability to reverse MDR when combined with vincristine or paclitaxel treatments both in vitro and in murine xenograft models [32]. Similarly, the canine MDCK cell line expressing BCRP2 was used to evaluate the activity of its quinazoline- and 4-methylpyrimidine-based inhibitors as potential tools to overcome MDR, by selecting the most promising compounds for in vivo applicability [33]. Recently, the MDR modulation in clinical field was addressed by using canine MDCK cell lines for evaluating brivanib, a novel TKI, to circumvent transporter-mediated resistance combined with classical cytostatic agents [34]. In particular, cell sublines overexpressing human ABC drug efflux transporters significantly increased the uptake of non-cytotoxic probes as well as of mitoxantrone and daunorubicin upon Brivanib exposure, partly reversing resistance to these cytostatic agents, without apparent development of resistance to brivanib itself [34].

Another current challenge in the fight against DR is the development of novel compounds able to counteract tumour progression and recurrence by targeting P-gp expressed by cancer stem cells (CSCs) rather than acting primarily on cell proliferation mechanisms. In this regard, MDCK-MDR1, MDCK-MRP1, and MDCK-BCRP cell lines allowed the identification of a novel specific P-gp ligand among tetrahydroisoquinoline derivatives. This compound demonstrated a potency comparable to that of elacridar and further enhanced doxorubicin chemosensitivity. Importantly, the efficacy of this P-gp inhibitor was confirmed in human CSC models derived from patients affected by glioblastoma and malignant pleural mesothelioma [35]. In addition, the P-gp inhibitory activity of crown ether compounds, an emerging class of anticancer agents, was tested with the help of MDCK-MDR1 cells, contributing to the design of novel molecules to reverse P-gp-related MDR in tumours cells [36]. Continued efforts to overcome some safety and efficacy issues with P-gp inhibitors may help improve the targeting of P-gp-mediated MDR in the future. Of course, future in vivo investigations on MDR could take advantage from these in vitro findings. Similarly, using different canine osteosarcoma cell lines as an in vitro model, it has been demonstrated that MDR1 is a molecular mechanism related to resistance to topoisomerase inhibitor etoposide, a member of a promising class of chemotherapeutic agents [37]. Human topoisomerase I, IIα, and IIβ have been recognized as potential targets for the development of novel anticancer inhibitory molecules for the treatment of diverse cancer types, and

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widely investigated in the updated literature. The relationship between the low expression of MDR1 and the clear susceptibility of cancer cells to topoisomerase inhibitors was supported by in vivo findings. In fact, the tumour growth in xenograft mouse models after subcutaneous implant of these cell lines was markedly reduced by etoposide treatment, in contrast to tumours induced by injecting canine mammary gland cancer cell lines in which MDR1 expression was upregulated [37]. These results suggest the usefulness of further investigation on the role of MDR1 as a predictive biomarker to evaluate outcome of treatment with topoisomerase inhibitor and MDR in comparative oncology. In further experiments, aimed at studying potential compounds capable of blocking the mechanism by which anticancer drugs are moved out of resistant tumour cells, MDCK cells and the variant expressing the human ABCB1 protein (MDCK-MDR1) were used to study the activity of isobavachalcone, a plant-derived molecule that has shown antiproliferative activity on several tumour cell lines [38]. Chalcone was shown to exert a higher cytotoxic effect on MDKC cells than on MDCK-MDR1 cells, suggesting its interference with the ABCB1 transporter. For validation, accumulation of the ABCB1 substrate Rho 123, was found to be twofold higher in MDCK cells compared to MDCK-MDR1 cells. In addition, chalcone treatment of MDCK-MDR1 cells combined with verapamil clearly reduced cell growth, confirming that it was a substrate of ABCB1 protein. These results suggested that the survival rate of MDCK-MDR1 cells was reduced by transporter inhibition, which in turn increased the accumulation of this active molecule inside tumour cells [38].

3.2. Cancer stem cells (CSCs)

The proliferation of CSCs in the contest of the tumour mass and their evident resistance to conventional chemotherapies contribute significantly to the development of DR and cancer recurrence in both canine and human patients. The CSC phenotype has been identified in cell populations of several canine and human tumours, including mammary, prostate and hepatocellular carcinomas, leukaemia, melanoma, glioblastoma and osteosarcoma, offering valuable translational models of complementary relevance to induced or xenograft rodent models.

Therefore, the identification of CSCs markers could aid in the development of novel translational therapies. Several comparative oncology studies have investigated the role of CSCs in canine and human osteosarcoma. Spontaneous canine osteosarcoma shows local invasiveness and frequent metastatic disease to lungs, with high tumour microenvironment heterogeneity and poor prognosis for treatment inefficacy similarly to the human one. Conventional chemotherapeutics such as methotrexate, doxorubicin and cisplatin are valid in both veterinary and human clinics for the standard treatment of osteosarcoma. However, at present, no new strategies are available for resistant or recurrent tumours in short-term follow-up, making investigations into the cellular mechanisms involved in DR of fundamental importance. Osteosarcoma cells may develop DR by impairment of drug uptake, enhanced DNA repair system activity, evasion of apoptosis, adaptive signalling from tumour microenvironment, and the presence of CSCs. In particular, the involvement of a small fraction of CSCs in tumorigenesis, as well as in treatment failure, has been clearly observed in both naturally occurring canine and human osteosarcoma, and in this regard similarities between canine and human cells have also been demonstrated [39]. Interestingly, Cyclooxygenase-2 (COX-2) overexpression was found in histological samples from both canine and human patients carrying osteosarcoma, suggesting a potential role of therapeutic strategies using COX-2 inhibitors. COX-2 is an inducible prostaglandin (PG) synthetase and PGs are likely to play a crucial role in angiogenetic and apoptotic processes in cancer. In this regard, COX-2 inhibition showed to have no effect on growth and DR of CSCs derived from both canine and human osteosarcoma cell lines, contrary to what was observed in daughter cells. Even though COX-2 expression was found upregulated in CSCs isolated from a canine patient with primary osteosarcoma, COX-2 inhibition using meloxicam and mavacoxib did not influenced growth and DR of CSCs in vitro, but it was able to prevent sphere formation from daughter cells, indicating a potential significant role for COX-2 in tumour initiation [39]. Similarly, the B cell-specific Moloney murine leukaemia virus integration site 1 (BMI1), a member of the Polycomb repressive complex 1 (PRC1) of transcriptional regulators, has been found to be highly expressed in tissue samples of primary and metastatic osteosarcoma from both dogs and humans. In particular, BMI1 seems to be involved in DR, suggesting that the dog may represent a valuable preclinical model to study the therapeutic potential of inhibition for this biomarker.

Indeed, BMI1 expression was found upregulated in both human and canine osteosarcoma cell lines, and its inhibition in vitro significantly reduced cell proliferation and increased sensitivity to

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carboplatin and doxorubicin in different canine lines. The overall results highlighted an interesting translational comparability between the tumour biology of human and canine osteosarcoma and a potential utility of targeting BMI1 in CSCs of this malignancy to overcome the challenge of DR in response to first-line chemotherapeutics [40]. The role of other transcriptional regulators such as hairy and enhancer of split-1 (HES1) in modulating CSCs maintenance and chemoresistance in osteosarcoma is still unclear. HES1 expression has been associated with increased aggressiveness in human osteosarcoma cell lines. Analysis of HES1 actions in primary canine osteosarcoma samples revealed that HES1 mRNA expression could be higher in tumour samples than in healthy bone tissue within individuals, while it was comparable to normal bone samples in dogs with faster tumour recurrence after treatment. HES1 expression was also found to be variable between cell lines in canine and human osteosarcoma cells. Overall, these findings suggest that other regulatory mechanisms may contribute to the aggressiveness of osteosarcoma and further researches in this field may provide new insights into therapeutic resistance in both canine and human cancers [41]. The fundamental involvement of CSC-promoting mechanisms has also been demonstrated in canine and human gliomas. Canine gliomas resemble human gliomas in several clinical, imaging and histological aspects, and similarities in various oncogenic signalling pathways have recently been discovered. The BMI1 protein have been found also involved in the development and DR of canine and human gliomas, through the generation of stem cells from astrocytes. The high expression of BMI1 protein in gliomas tissue sample from canine patients, as well as the anti-proliferative effects of BMI1 inhibition by the PTC-209 molecule in several cell lines cultured from spontaneous canine gliomas has been clearly evidenced, supporting the hypothesis of a potential utility of comparative studies to test new therapies, with benefits for both species. These findings suggest that spontaneous canine gliomas may represent an advantageous translational model, overcoming challenges related to experimental systems induced in laboratory rodents, for example to study novel treatments that can cross the BBB within a human-like vascular tumour microenvironment [42]. Molecular characterization of primary cultures of CSCs isolated from post-surgery canine osteosarcoma specimens as well as by immunohistochemistry from tumour fixed sections, has highlighted analogies with humans on overexpression of chemokine receptor type 4 (CXCR4) and its chemokine ligand type 12 (CXCL12) [43]. This intracellular signalling pathway seems to be involved in osteosarcoma CSCs proliferation and migration in metastatic sites in both dogs and humans. By acting on this mechanism, the oral hypoglycaemic drug metformin has been shown to increase the cytotoxicity of a combined treatment with doxorubicin and cisplatin in an in vitro canine osteosarcoma model, similar to the results described on human osteosarcoma cell cultures [43]. Considering that similar results have also been reported in stem cells derived from canine mammary carcinoma, human hepatocellular carcinoma or human glioblastoma, the CXCR4/CXCL12 axis could represent a promising target to overcome the problem of DR, and results obtained in veterinary oncology may represent a preclinical starting to future improvement of human therapeutic strategies. The impact of metformin on the mechanisms of resistance to anticancer drugs has also been studied on a canine model of B-cell lymphoma, highlighting its role on the activity of the Anaphase Promoting Complex (APC), a molecular pathway evolutionarily conserved in living organisms. APC has been found to be altered in dogs carrying DR lymphomas and in vitro using doxorubicin-resistant canine lymphoma cell lines. After administration of metformin in combination with the conventional protocol including cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulphate, and prednisone, canine patients with recurrent DR lymphomas showed decrease MDR proteins comparable to those found elevated in many cases of MDR cancers in humans, including MDR-1. Furthermore, APC activation was observed, leading to partial remission and clinical benefits, and this effect was confirmed by restored chemosensitivity using in vitro tests [44]. This canine model suggested that impaired APC activity may be a marker of MDR associated with poor prognosis, and that APC may represent a promising target in the prevention of anticancer DR in patients. Metformin has also been shown to suppress CSCs survival in vitro as well as in preclinical models of breast cancer [45]. Spontaneous mammary tumours have high incidence in female dogs, representing a valuable opportunity for comparative oncology research given the genetic, environmental, clinical and molecular similarities to human breast cancer. In this regard, CSCs isolated from canine mammary carcinoma showed molecular characteristics and resistance to antineoplastic agents similar to their human counterpart. Compared to continuous cell lines, these CSCs derived from spontaneous, non-treated canine mammary cancer advantageously represent the

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tumour biology and heterogeneity in vivo, even when implanted in mouse models, thus improving the knowledge provided by experimentally induced cancer in laboratory rodents. Overall, these aspects strongly spotlight the translational validity of new anticancer drugs tested in preclinical models and show up that their optimization improves the identification of clinically useful compounds in humans. CSCs derived from canine mammary tumours have shown resistance to doxorubicin, mainly related to the involvement of ABC transporters in limiting the intracellular accumulation of the drug, as demonstrated by their inhibition by verapamil. Conversely, metformin significantly reduced CSCs proliferation both in vitro and in immunodeficient xenograft mice models, as assessed by antigen Kiel 67 Labelling Index (Ki-67-LI), a biological marker for several human and canine cancers [45]. These findings could be helpful in developing successful therapeutic strategies targeting breast cancer CSCs for the benefit of both veterinary and human medicine. Similarly, canine prostate cancer closely resembles human one in clinical progression and histopathological features, and CSCs play a pivotal role in its therapeutic resistance. Preliminary in vitro studies using the canine prostate adenocarcinoma cell line CT1258 show mild doxorubicin resistance, high metabolic activity, and expression of the alpha-6 integrin that characterizes tumour cells with stem-like characteristics. These findings represent a promising translational information to further characterize the role of CSCs in prostate cancer and discover potential therapeutic targets through in vivo studies [46]. Recently, sex determining region Y-box 2 (SOX2) has been identified as a crucial regulator of CSCs pluripotency in different tumours of humans and rodent models, and has been therefore correlated to DR and poor prognosis. In a comparative oncology investigation, SOX2 overexpression was also found in tissue samples from many types of canine neoplasia. Although the role of this CSC marker in each tumour histotypes remains to be better understood, these findings may provide useful insight into DR mechanisms and help guide new translational perspectives to address this relevant challenge in comparative oncology [47].

Among the new generation targeted therapeutic strategies to overcome intrinsic or acquired resistance of tumor cells to conventional cytotoxic agents or radiotherapy, bispecific ligand-targeted toxins (BLTs), designed to bind specific receptors expressed by tumor cells, have been employed, with limited toxic side effects.

Interestingly, highly chemotherapy-resistant Emma, Frog and SB cells derived from canine hemangiosarcoma, including CSC-enriched cultures, have demonstrated an improvement in cytotoxicity and safety of a newly synthesized deimmunized Pseudomonas exotoxin conjugated to epidermal growth factor and urokinase (EGFuPA toxin), which are overexpressed in several tumours and particularly in sarcomas and tumour endothelial cells.

Canine hemangiosarcoma represents a suitable model to study highly resistant sarcoma, very similar from a molecular point of view to human angiosarcoma and, in particular, a tumour stem cell platform in which CSCs play an important role in contributing to chemoresistance [48]. Overall, these data highlighted the utility of the canine hemangiosarcoma model to develop novel approaches to circumvent DR, suggesting integrative fields for cancer treatment, particularly in tumours where increased resistance to conventional cytotoxic drugs has been associated with CSCs.

3.3. Epigenetic Alterations

Epigenetic modifications of DNA, such as methylation, histone modification, or chromatin remodelling, can upregulate the expression of oncogenes, induce increased drug efflux, enhance DNA repair, or impair apoptosis, resulting in DR. Methylation has been linked to chemoresistance in canine lymphoma, making this animal model an attractive candidate for testing demethylated drugs for use in both chemotherapy-resistant canine and human patients. Among drug-resistant cellular methylation models, hypermethylation of the CpG island in the region upstream of exon 2 in the ABCB1 gene was found in drug-sensitive canine lymphoma cell lines, in contrast to hypomethylation in drug-resistant ones, as confirmed in vivo. Similarly, methylation of other gene promoter regions has been found to be associated with resistance to l-asparaginase (L-asp) [49], as well as to lomustine or doxorubicin [50], in canine lymphoma cell lines. In particular, the canine lymphoma cell lines OSW and CLGL-90 have been useful in further elucidating the mechanisms related to L-asp resistance in lymphoma, demonstrating that epigenetic regulation of asparagine synthetase expression by methylation of its promoter was inversely related to mRNA or protein levels and directly to L-asp resistance [49]. However, asparagine synthetase methylation showed a variable status in canine patients with high-grade B- and T-cell lymphoma and was not significantly correlated with clinical

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outcomes. Furthermore, the asparagine synthetase promoter was found to be overall hypomethylated in both human lymphoma cell lines and tissue samples. Such contradictory results between in vitro and in vivo data suggest the need for further studies to evaluate the utility of L-asp in translational therapy of lymphoma [49].

3.4. Apoptosis and Epithelial–Mesenchymal Transition (EMT)

Cancer cells are more resistant to apoptosis, a form of cell death triggered by exposure to various cellular stresses. This biological characteristic not only allows the tumour to become more aggressive but also contributes to DR. Exploring the scientific literature, the canine model seems particularly relevant to study aberrations in gene expression involved in anti-apoptotic mechanisms and associated with DR in both humans and dogs. Furthermore, some reports of potential therapies targeting these mechanisms in both species suggest their possible efficacy and prospects for future researches. B-cell lymphoma is the most common hematopoietic malignancy in both dogs and humans and shares many biological, genetic, and molecular characteristics, including the Feline McDonough Sarcoma (FMS)-like tyrosine kinase 3 (FLT3) mutation. In a constitutively active state, this oncogene targets several downstream proteins such as the signal transducers and activators of transcription (STAT), mitogen-activated protein kinase (MAPK), and protein kinase B (AKT) pathways leading to cellular proliferation and resistance to apoptosis [51]. Using genomic polymerase chain reaction (PCR), conserved FLT3 mutations between species have been demonstrated in both the canine GL-1 cell line and in clinical specimens of canine B-cell lymphoma. Although FLT3 mutations found in canine models show differences in frequency of occurrence and prevalence in distinct histotypes compared to the human counterpart, the major downstream-targeted pathways, STAT5 and extracellular signal-regulated kinase (ERK)1/2 proteins, appeared overall evolutionarily conserved. Furthermore, the canine GL-1 cell line was found to be sensitive to the FLT3 inhibitor lestaurtinib, similar to the human leukaemia cell line MV4-11 carrying a comparable FLT3 mutation [51]. Overall, these findings highlight that both cellular and clinical canine models of leukaemia may be appropriate to study common mechanisms of oncogenesis, relevant to both species. FLT3 appears to be a promising therapeutic target, in light of its upregulation in several forms of acute leukaemia, and a deeper understanding of FLT3 mutations through valid experimental models could improve the development of more effective FLT3 inhibitors in overcoming DR. Similarly, activation of the B-cell nuclear factor kappa-light-chain-enhancer (NF-kB) pathway has been shown to play a central role in MDR by promoting cell proliferation and exerting antiapoptotic effects. In companion dogs with spontaneous B-cell lymphoma, comparative overexpression and constitutive activity of NF-kB have been found [52]. Furthermore, intranodal administration of the IK kinase (IKK) complex-selective peptide inhibitor of the essential modulator-binding domain of NF-kB (NEMO) in canine patients with relapsed B-cell lymphoma was able to inhibit NF-kB expression and reduce tumour burden. In vivo findings, together with in vitro induction of apoptosis in primary malignant B cells, highlighted for the first time the translational relevance of NF-kB inhibition in the treatment of B-cell lymphoma [52]. In a following study, improved methodologies were implemented to work with canine primary cells, including optimized procedures for isolating lymphocytes from canine malignant lymphoid tissue, treating them with novel anticancer drugs, and assessing signalling pathways relevant to proliferation, survival, and DR. Taking advantage of these advances, the therapeutic potential of the NEMO-binding domain peptide was studied in primary canine malignant B cells in vitro, showing the ability to induce apoptosis by blocking NF-κB signalling via inhibition of the upstream IKK regulatory complex [53]. Overall, available data of aberrant NF-kB signalling in different canine cancers, and on the potential to inhibit NF-kB activity by using antineoplastic compounds suggest that dogs may be a suitable model for comparative cancer biology studies on regulatory mechanisms of NF-kB leading to DR in human cancers [54]. Spontaneous canine mammary tumours show a clinical evolution and molecular features similar to those of human breast cancer. Canine mammary gland cancer cell lines appear to be of great value for translational research on tumour microenvironment, treatment and DR in breast cancer. The canine mammary cancer cell line B-CMT established from a primary mammary adenocarcinoma diagnosed in a female dog, has been described as a triple-negative cell line that overexpresses hypoxia inducible factor-1α (HIF-1α), leading to doxorubicin resistance by inhibition of apoptosis and P-gp overexpression. Furthermore, the B-CMT cell line appears to be able to replicate the biological characteristics of the primary tumour in immunodeficient mouse xenografts [55]. These features could be of great significance to provide further insights into future researches including that on

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MDR. Recently, the effect of celastrol, an extract of the plant Tripterygium wilfordii, an alternative treatment in traditional Chinese medicine for cancer, was comparatively studied on canine mammary tumours, aiming to improve treatment options in canine and human patients [56].

In particular, celastrol has been shown to inhibit NF-kB and B-cell leukemia/lymphoma 2 (Bcl-2) signalling pathways, as well as increase the expression of Bcl-2 associated X (Bax) protein and caspase, involved in the induction of apoptosis of several types of tumour cells, including breast cancer. In vitro proliferation and migration ability of the triple-negative canine mammary gland cancer cell line CMT-7364, as well as cell line CIPp, were selectively inhibited by celastrol treatment in a dose-dependent manner, acting through a dual mechanism by inducing caspase-mediated cell apoptosis and cell cycle arrest by regulating cell cycle–related proteins. Considering that consistent results were found in human triple-negative breast cancer cell lines, this study provided a proof of concept on the anticancer mechanisms of celastrol and for its potential clinical application [56].

Gene expression analysis in primary human tumours has emerged to identify biomarkers of chemotherapy resistance and pathways related to treatment response. In this regard, comparative analysis of gene expression profiles has highlighted marked similarities between human and canine osteosarcoma. In particular, a molecular screening of poor- versus good-responder canine osteosarcoma patients revealed that the Hh signalling pathway plays a crucial role in both osteosarcoma progression and therapy resistance, primarily through inhibition of apoptosis [57]. Although the overall mechanisms by which Hh signalling promotes DR are still under investigation, understanding them is relevant to the development of effective treatment strategies. The results of this study suggested the potential usefulness of comparative genetic and molecular prognostic screening between humans and dogs to translate novel insights into the mechanisms of osteosarcoma progression and chemoresistance. More recently, enhanced cell proliferation and reduced apoptosis, leading to DR, were comparatively found in several human and canine osteosarcoma cell lines related to overexpression of ephrin A2 (EphA2) receptor tyrosine kinase. Upregulation of EphA2 in both human and canine osteosarcoma cells supported migration and invasiveness in vitro, likely mediated by integrin β3 expression, as confirmed by EphA2 silencing [58]. Consistent with these findings and based on the same comparative oncology approach, suppression of EphA2 activity was found to significantly reduce tumour growth in xenograft mouse models of implanted canine osteosarcoma cells. Furthermore, increased EphA2 expression induced resistance of osteosarcoma cells to cisplatin in both species, probably through the activation of the Rous sarcoma proto-oncogene (cSRC), AKT and/or ERK–MAPK pathways, as confirmed by the reduction of their phosphorylation following EphA2 silencing [58]. Overall, the similarities in EphA2 receptor expression and function in both species suggest that it may represent a promising therapeutic target both to overcome MDR in osteosarcoma and to potentially reduce the side effects of cisplatin in combination therapies. The translational potential of these findings could benefit from the shorter lifespan and faster progression of osteosarcoma in canine patients compared to humans, improving knowledge of treatments with benefits for both species. Although there are numerous similarities in the mechanisms of DR in various canine and human tumours, it is important to recognize that species-specific differences have also been found. Aurora kinases A and B (AURKA, AURKB) are involved in cell mitosis and are overexpressed in several human malignancies, thus representing promising therapeutic targets. Some canine osteosarcoma cell lines highly express Aurora kinases, but treatment with their inhibitors failed to consistently increase apoptosis compared to the effects exerted on human tumours that overexpress these kinases, highlighting differences in the potential utility of these drugs in veterinary and human cancer therapy [59]. In tumour cells, telomerase activity and maintenance of telomere stability are associated with increased cellular resistance to apoptosis. For this reason, several antitumor strategies have been studied that inhibit the telomerase enzyme, causing DNA damage and programmed cell death in experimental models. In this regard, the effect of telomerase inhibition by small interfering RNA (siRNA) and short hairpin RNA (shRNA) oligonucleotides was evaluated in canine hemangiosarcoma SB/HSA.2 cells [60]. The study results suggested that telomerase could be a potential target for anti-cancer therapies in dogs, but measurements of telomerase expression also showed that these cells develop resistance to inhibition of this enzyme over time. Subsequent in vivo tests in murine xenograft models of canine hemangiosarcoma inoculated with early- or late-passage SB/HSA.2 cells demonstrated that telomerase inhibition could effectively target the growth of canine hemangiosarcoma, but there is also the possibility that resistance may occur. Overall, these findings suggest that natural canine cancer models may be useful

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in bridging the translational gap between human patients and mouse models in the study of telomerase-based therapies [60].

Epithelial-mesenchymal transition (EMT), a cellular process that tumour cells can undergo, plays a crucial role in tumour recurrence and DR-related metastasis, representing a relevant clinical problem in prostate cancer therapy. Several regulatory mechanisms are involved in EMT, and resistance to apoptosis of cells detached from the surrounding extracellular matrix is among the mechanisms through which EMT may contribute to anticancer DR. Spontaneous canine prostate cancer has proven to be a valuable model of androgen-independent prostate cancer in humans, as well as the canine Ace1 cell line, derived from a primary canine prostatic carcinoma, which shares similar signalling pathways and receptors upregulated in the human counterpart. Using this cell model, AR-42, a promising histone deacetylase inhibitor, was shown to reduce tumour cell proliferation in vitro primarily by inducing apoptosis, and in vivo intracardiac injection of Ace-1 cells into nude mice demonstrated that AR-42 was able to prevent bone metastasis of prostate cancer [61]. In this perspective, future studies evaluating the effects of new histone deacetylase inhibitory anticancer drugs on other human and canine prostate cancer cell lines could be useful to develop therapies more effective to target EMT- and apoptosis-related resistance, in turn counteracting prostate cancer bone metastasis.

Overexpression of epidermal growth factor 2 (HER2) in women, together with the lack of steroid hormone receptors, is associated with more aggressive breast tumor growth and acquisition of DR through major pathways such as the wingless-type MMTV integration site (Wnt) family or the Rous sarcoma proto-oncogene (cSRC). Interestingly, the canine mammary tumor (CMT)-U27 cell line showed high basal Wnt activity and high levels of EGFR, HER2, and HER3 mRNA expression, similar to HER2-overexpressing human luminal cell lines. These cells are characterized by an EMT phenotype, suggesting that inhibition of Wnt activity could influence breast cancer malignancy in some histotypes. Indeed, inhibition of Wnt activity led to reduced invasiveness of CMT-U27 cells. Furthermore, highly activated Wnt signaling in the canine mammary cell line CMT-U27 was not found to correlate with HER2/3 signaling by siRNA silencing, nor with cSRC activation, as demonstrated by in vivo experiments using the CMT-U27 xenograft in zebrafish embryos treated with the cSRC inhibitor dasatinib [62]. These results suggest the need of further studies to elucidate the complex interaction of signaling pathways in DR to HER-inhibitors in HER2/3-positive breast cancer.

3.5. Drug Target Alteration

Compared to conventional cytotoxic agents, selective targeted therapies have the advantage of interfering with tumour growth by inhibiting the activity of specific proteins with fewer side effects on normal cells.

Several genetic mutations involving chemotherapeutic targets, such as tyrosine kinases and topoisomerases, lead to therapeutic resistance through alterations in molecular signalling pathways or binding.

Recent advances in genomic and proteomic analyses have contributed to their increasing identification in both humans and canine species.

Over the past decades, these mechanisms have been studied fragmentarily in various canine cell lines and in natural tumours.

Signal transduction between the HER1 and HER2 transmembrane tyrosine kinases and cell nuclei is mediated by RAS proteins, and mutations in its gene KRAS are associated with resistance to conventional monoclonal antibodies that target those proteins. The role of this molecular pathway has been widely recognized in human gastric cancer and, interestingly, HER1 and HER2 were found overexpressed in several canine gastric tumour samples. Although the DNA sequence of the canine KRAS gene is very similar to that of humans, KRAS mutations were uncommon in the canine patients analysed. Nevertheless, single mutation of codon 12 is the most frequent in humans and could similarly determine DR in dogs by altering the inhibitor/target interaction and preventing binding [63]. Although gastric tumours in dogs closely resemble human clinical and histological features, their incidence is rare.

Further studies on the role of HER1/HER2/KRAS pathways in canine oncology could help to understand the utility of this comparative model to address DR both canine and human patients [63]. Instead, a comparative analysis of melanoma cell lines derived from primary tumours or metastasis

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of human, canine and equine species showed comparable mutations in proto-oncogenes BRAF, NRAS and KIT, which are well-known factors regulating proliferation and apoptosis in human melanoma [64]. Indeed, the inhibitor LY3009120, which targets these pathways in both cells with and without RAS mutations, has been proposed as a promising therapeutic approach in comparative oncology, with the advantage of minimal paradoxical activation of ERK signalling compared to BRAFV600E-selective inhibitors, thereby reducing the risk of unwanted enhanced tumour growth. Significant growth inhibition was observed in nearly all melanoma cell lines after exposure to LY3009120, suggesting its value for further in vitro and in vivo testing to target BRAF-resistant tumours and to study resistance mechanisms in comparative oncology [64]. Interestingly, sequencing analysis of BRAF in canine transitional carcinoma cells revealed close homology to human BRAF and that its mutations commonly result in increased MAPK pathway activity in both human and canine tumours. In addition, upregulation of receptor tyrosine kinases has been found among the mechanisms of resistance to BRAF inhibition. In this regard, in vitro growth of several BRAF-mutated canine transitional carcinoma cell lines was not inhibited by the BRAF inhibitor vemurafenib, whereas MAPK inhibitors do so similarly to human BRAF-mutant cell lines. However, monotherapy of such cell lines with BRAF or MAPK inhibitors leads to a temporary attenuation of MAPK pathway activity, while combination treatment with EGFR receptor inhibitors has been shown to act synergistically [65]. Taken together, these data suggest the utility of canine transitional carcinoma as a model to investigate the role of MAPK pathway in modulating cancer progression and acquired DR.

Similarly, canine B-cell lymphoma represents a valuable model of the homologous human disease, with the advantage of an immunosuppressive tumour microenvironment over immunocompromised murine xenograft models. DR occurs in both species and, therefore, studying immunotherapy in dogs with spontaneous B-cell lymphoma has been proposed as useful for identifying mechanisms of resistance to chimeric antigen receptor (CAR)-T cell therapies [66]. Resistance to CAR-T cell therapies occurs in most human patients by loss or downregulation of CD19 and/or CD22 on malignant B cells leading to “antigen escape”, or expression of inhibitory ligands, such as programmed cell death 1 ligand 1 (PDL1), impaired apoptosis and an immunosuppressive tumour microenvironment [66]. Overall, these mechanisms need to be studied using appropriate experimental models to develop more effective therapeutic strategies. The development of tumour resistance to CAR-T cells that target a single antigen is very common. A methodological study on the implementation of CAR-T cell therapy in canine patients demonstrated that canine T cells, modified to express a chimeric antigen receptor specific for CD20, were able to cause cell death in canine and murine B-cell lymphoma cells expressing canine CD20, providing further novel data to improve their role as models of cancer resistance [66].

Still, canine mast cell tumours have been suggested as a useful model of altered function of Kit, a receptor tyrosine kinase present in various human and spontaneous canine cancers, for evaluating the efficacy of new inhibitory molecules [67]. Mutations in Kit have been found to induce its constitutive activation, promoting uncontrolled cell proliferation and survival. Furthermore, secondary mutations after monotherapy with specific inhibitors frequently lead to DR in the clinical setting, for example toward imatinib, sunitinib or dasatinib, due to altered drug binding and/or activity. The expression of this oncogenic protein is regulated by heat shock protein 90 (HSP90), therefore, its targeting with inhibitors would degrade this protein and act as a valuable antitumor strategy.

In this regard, the effect of STA-9090, a heat shock protein (HSP) 90 inhibitor, was evaluated in canine malignant mast cells with different mutations of Kit. STA-9090 reduced proliferation and viability of malignant mast cell lines as well as malignant mast cells cultured from histological samples, and it was able to inhibit tumour growth in vivo in a murine xenograft model [67]. This study suggests the opportunity for future clinical studies to test novel treatment strategies using HSPs inhibitors in canine patients with spontaneous cancers, to gain new insights useful to clinical application in humans.

3.6. DNA Damage and Repair

Among the different mechanisms of resistance to therapy developed by tumour cells, one of the most important is intratumoral heterogeneity. DNA repair pathways play an important role in maintaining genome stability, and defects in related mechanisms contribute to tumour heterogeneity,

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leading to the selection of cellular sub-clones, mutations, and resistance to single-agent chemotherapy. Chemotherapeutics that target DNA repair pathways have been explored, however resistance to these drugs is also being found and studied, and represents a growing problem in the clinical setting. In particular, osteosarcoma shows comparable genetic complexity and histotype heterogeneity in humans and dogs, which favour recurrence and DR and, therefore, affected individuals still show limited survival despite scientific advances in cancer treatment.

Osteosarcoma represents therefore a promising candidate for personalized medicine approaches, and dogs have proven to be a valuable translational model for this cancer, helping to fill gaps in knowledge of DR and identify potential therapeutic targets for future clinical trials. Canine patients with spontaneous osteosarcoma usually undergo limb amputation prior to chemotherapy, allowing comparisons of the genetic profile between normal bone and tumor tissue sampled in the same subject and avoiding interpretation problems that occur in human patients related to acquired DR. In this regard, several human homologous genes resulted upregulated by comparing their expression in primary canine osteosarcoma and normal tissues collected from the same chemotherapy-naïve subject. Among them, the GTSE1 gene was identified, that regulates the cell cycle and may induce cisplatin resistance in human osteosarcoma through inhibition of p53, in turn blocking apoptosis in tumor cells in response to DNA damage [68]. More recently, the same research group conducted another comparative study on osteosarcoma, focusing on the role of tumor heterogeneity in the evolution of different cellular subsets that promote DR.

A primary canine osteosarcoma specimen before any therapy was characterized, showing significant genomic instability and chromosomal copy number variations, as well as clusters of osteoblasts, fibroblasts, endothelial cells, myeloid cells, and CD4+ T cells. These findings were consistent with those found in human patients, suggesting useful interspecies similarities in osteosarcoma heterogeneity [69].

4. Discussion and Conclusions

The complexity of cancer makes it advantageous to use a multidisciplinary approach and the combination of different state-of-the-art study tools and models in patient care.

Despite ongoing efforts to develop effective chemotherapeutics, DR in cancer currently represents the major limitation to patient survival.

Hence, the development of new treatment strategies overcoming MDR is an urgent need.

Canine cancer models have the advantage of representing a genetically broader population than inbred mouse models, and of sharing similar responses to the same chemotherapeutics as humans, better addressing some scientific questions related to preclinical drug development and cancer resistance [10].

The literature on the mechanisms of DR in dogs with cancer and those shared with humans consists primarily of case reports scattered throughout the literature.

Therefore, we provided a comprehensive review of all currently known DR pathways found in common between humans and dogs with cancer, possible strategies to overcome them by targeted therapies, and a reflection on future perspectives.

The purpose of this review was to evaluate the potential utility of DR overlap between dogs and humans, providing evidence of the value of the canine model in translational research. The results of the reviewed studies showed that both canine cancer cell lines and dogs bearing spontaneous tumours represent powerful and still underutilized experimental models to develop new knowledge on chemotherapy and related DR [10].

From the past until today, most cancer research has been conducted on mouse models, mainly because of their easy handling and genetic manipulation, and relatively low breeding costs.

However, they have shown some limitations in mimicking human tumours, resulting in only a small fraction of the new drugs tested progressing to clinical trials and being approved for use in patients.

Connecting animal and human medical research outside the laboratory, in a One Health perspective, can help strengthen collaboration and coordination in cancer investigation.

To promote translational studies, it is important highlight the relevance of experimental results and share them among researchers from different fields of life sciences.

Furthermore, citizen science projects, which involve the public in canine scientific research, could usefully integrate data collection into established experimental methods.

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The bibliometric analysis of scientific articles selected from our literature search revealed a clear increase of researches about the DR mechanisms shared by canine and human cancer patients over the years.

Overall, Figure 3 illustrates the annual scientific production on the DR mechanisms shared by canine and human cancer patients from 1990 to 2024.

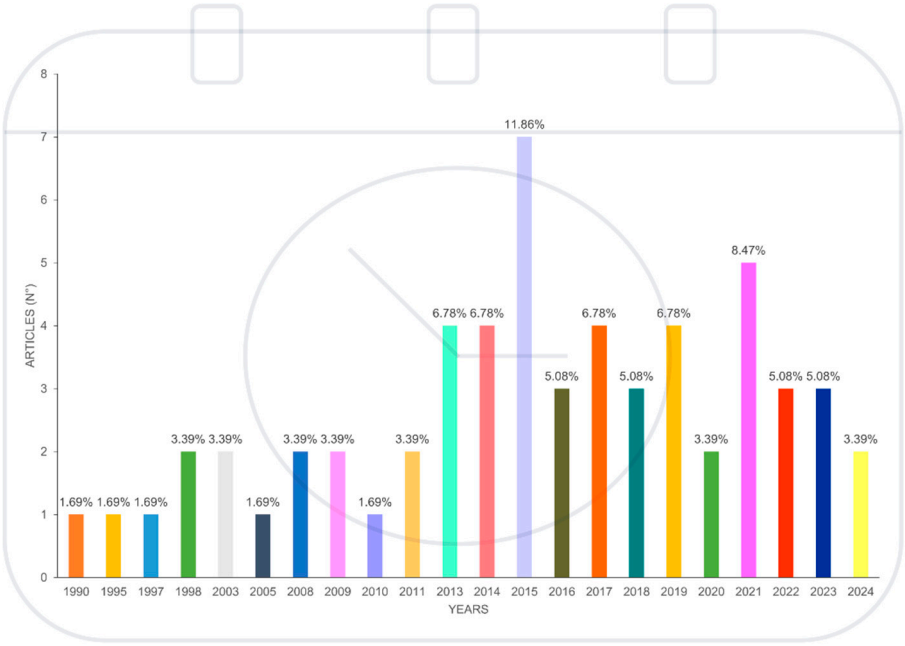

Figure 3. Annual scientific production.

Initially, from 1990 to 2000, the number of articles published annually on this topic remained relatively low, fluctuating between 1 and 2 articles per year. A notable increase begins in 2013 and continues until 2024, with the number of articles on this topic consistently reaching 4 articles per year and peaking in 2015 and 2021, with 7 and 5 articles per year, respectively. Overall, this data demonstrate a growing interest in this topic, which translates in an increased research output, although in recent years the COVID-19 pandemic has likely shifted the focus of One Health research away from comparative oncology. Several countries have shown increasing sensitivity to the One Health approach in oncology, as highlighted in Figure 4, where scientific production by country is expressed as a percentage of the total articles found.

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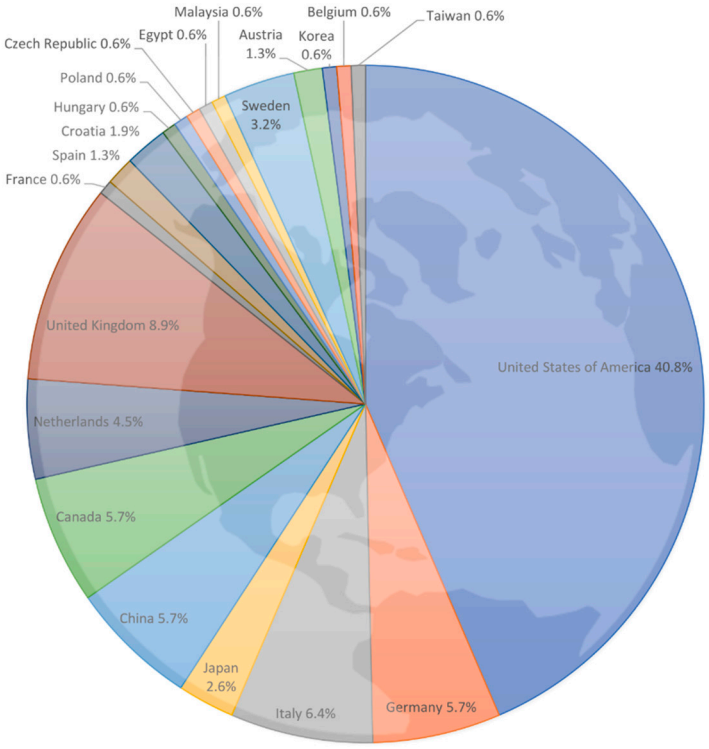

Figure 4. Scientific production by country.

The United States is actively working on One Health approaches in oncology, with 40.8% of articles, followed by the United Kingdom with 8.9% of articles, in line with the historical leadership that these countries have had in the progress and ethics of veterinary medicine. Italy has also demonstrated a strong involvement in this research area, covering 6.4% of the articles. Germany, Canada and China contributed almost equally with 5.7% of the articles, demonstrating significant participation in research on this topic. Netherlands, Sweden, and Japan have produced articles in a range from 4.5 to 2.6 % and overall, other 14 countries across Europe, Africa and Asia have produced articles in a range from 1.9 to 0.6 %, underlining the widespread international commitment to advancing knowledge and addressing oncology issues through a "One Health" approach.

The study of spontaneous tumours in companion dogs represents a valuable opportunity for research into new therapies with benefits for veterinary and human oncology. Advances in the genomic characterization of numerous types of canine tumours have facilitated the implementation of precision medicine to treat dogs. Importantly, given the natural occurrence of tumours in dogs sharing the human environment, the intra- and inter-individual complexity of cancer and resistance patterns can be more adequately represented compared to traditional research models.

Furthermore, some homologous tumours, which can be extremely aggressive and highly resistant to conventional therapies, occur spontaneously in companion dogs at a higher frequency than in humans.

This could have important clinical implications, providing an alternative source of valuable samples for comparative studies on challenges faced by humans, enabling to translate precision oncology implementation into human patient trials.

Therefore, it is advisable, for clinical and research purposes, to standardize the collection of tumor material, as well as implement molecular analyses on large pools of canine breeds, to broaden the knowledge of the genetic landscape, resulting in further strengths for precision medicine.

Additionally, there are relative ethical concerns in treating canines with naturally occurring cancers compared to induced cancer models, and simpler regulation in veterinary medicine, including an informed owner's consent, allows for easier testing of modified conventional chemotherapy protocols and off-label drugs [70].

In particular, the One Health concept provides an integrative perspective on public health, but still carries with it an intrinsic criticality from an ethical and regulatory point of view and is currently finding its place in legislation.

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The “One Medicine, One Health" approach is based on the idea that human and veterinary medicine should contribute to each other.

Inevitably, the status of “sentient beings” recognized to animals by European legislation influences the One Health vision.

However, a utilitarian and anthropocentric perspective continues to be mainly supported rather than promoting the One welfare vision on the direct and indirect benefits of improving animal wellbeing, focusing on the prevention of pandemics, antimicrobial resistance, zoonoses and emerging infectious diseases [71,72]. Nowadays, the concept of One Health has become much broader and could benefit from the integration of comparative oncology, translating knowledge on cancer resistance mechanisms into new prevention and treatment strategies in human patients [73]. In this perspective, the inclusion of translational studies on spontaneous canine tumour models in the preclinical evaluation of new anticancer therapies has been proposed as “proof of principle” investigations. However, several challenges hinder the effective implementation of the “One Medicine, One Health” strategies, including still limited communication between veterinary and human health professionals, as well as other scientific researchers, and poor collaboration between interdisciplinary infrastructures [74]. Although veterinary clinical trials may be useful to improve drug development and address the problem of DR, taking advantage of the short lifespan of dogs and consequently the earlier evaluation of drug activity, their clinical validity has sometimes been questioned, due to the limited standardization of the study design.

Overall, we interpret these data to suggest that a deeper understanding of cancer biology in dogs and humans may be helpful in gaining broader acceptance of preclinical natural models of canine cancer in the scientific community. They could valuably complement current induced rodent or xenograft models, addressing specific questions and ultimately benefiting both species. A conceptual framework for implementing a One Health approach in the study of comparative oncology and chemotherapy resistance should include legislation, funding, promoting future collaborations between human, veterinarian and environmental institutions, linking the One Health vision to the 3Rs principle in animal experimentation and pursuing a culture of care approach.

The implementation of the One Health approach highlights how progress for one species can also influence others.

This review shows that humans and dogs can complement each other in the study of cancer pathogenesis and therapy. A One Health approach, including human and canine patients, would represent a valuable joint effort and offer opportunities to accelerate knowledge, promoting better survival of humans and dogs. The institutional authorship of the examined scientific production is shown in Figure 5 and expressed as a percentage based on the total number of affiliations found in the articles.

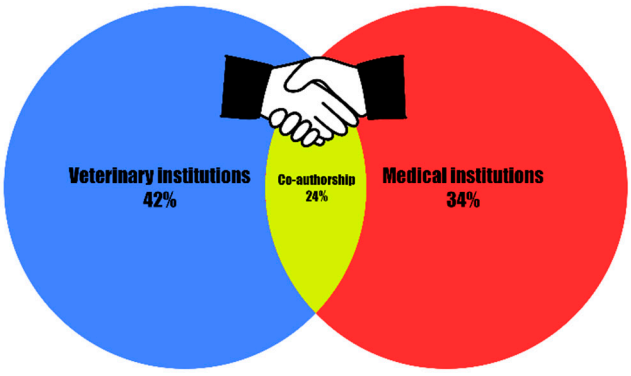

Figure 5. Authorship of scientific production.

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Veterinary clinical and research institutions provided the largest contribution (40.8%) compared to medical institutions, the latter mainly focused on evaluating the molecular mechanisms of DR in canine cellular models or using them to test new pharmacological compounds (34%). Interestingly, 24% of scientific research showed a co-authorship between medical and veterinary institutions, underscoring the importance of transdisciplinary cooperation in addressing complex health challenges such as chemotherapy resistance. Today, advances in comparative oncology knowledge and available diagnostic tools for early cancer detection in companion dogs, such as liquid biopsy, innovative imaging, and genomic and molecular testing, could enable broader translational benefits toward precision medicine across species. On the other hand, the economic accessibility and specialized expertise to these diagnostic technologies hinders their widespread application in veterinary oncology, comparable to the standards of human healthcare, with differences between countries around the world [75].

Furthermore, the cost-benefit ratio between welfare and life expectancy in dogs with cancer and concerns about the extra-time and financial efforts required of dog owners, together with the acceptance of an uncertain success rate of therapies, may limit informed decisions about treatment modalities to palliative ones [76,77].

Therefore, implementing collaborative research activities with academic or community nonprofit organizations for cancer prevention and early diagnosis, coupled with policy efforts and comparative oncology training programs, could provide an interdisciplinary strategy to disseminate a cross-species utilitarian view and promote information on DR from natural tumor models for the benefit of humans and companion dogs.

We are confident that interactions between researchers studying human and animal health will become increasingly common and that collaborative efforts across disciplines will be encouraged, promoting scientific progress in accordance with the modern "One Medicine, One Health" perspective. We believe that initiatives that enhance interdisciplinarity will be useful in developing new treatment strategies for DR in cancer patients.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, RM file.

Author Contributions: Conceptualization, methodology, project administration, data curation, writing—original draft preparation, S.G; formal analysis, resources, graphical editing, M.G.; writing—review and language editing, critical review, L.V.; critical review, supervision, E.P. All authors have read and agreed to the published version of the manuscript.

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Abbreviations.

The following abbreviations are used in this manuscript:

WOS	Clarivate Web of Science
MEDLINE	Medical Literature Analysis and Retrieval System Online
DR	Drug resistance
CCR	Cancer Institute's Center for Cancer Research
COP	Comparative Oncology Program
OSF	Open Science Framework
ABC	ATP-binding cassette
P-gp	P-glycoprotein
MDR1	multidrug resistance 1
MRP1	multidrug resistance-associated protein 1

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MRP2	multidrug resistance-associated protein 2
BCRP	Breast Cancer Resistance Protein
MDR	multidrug resistance
BBB	blood-brain barrier
CNS	central nervous system
Rh123	rhodamine123
MDCKII	Madin-Darby canine renal epithelial cell line
CSCs	cancer stem cells
Hh	Hedgehog
PARPs	polymerases
EGFR	epidermal growth factor receptor
TKI	tyrosine kinase inhibitor
COX-2	Cyclooxygenase-2
PG	prostaglandin
BMI1	Moloney murine leukaemia virus integration site 1
PRC1	Polycomb repressive complex 1
HES1	hairy and enhancer of split-1
CXCR4	chemokine receptor type 4
CXCL12	chemokine ligand type 12
APC	Anaphase Promoting Complex
Ki-67-LI	Kiel 67 Labelling Index
SOX2	sex determining region Y-box 2
BLTs	bispecific ligand-targeted toxins
EGFuPA	epidermal growth factor and urokinase
FMS	Feline McDonough Sarcoma.
L-asp	l-asparaginase
FLT3	FMT-tyrosine kinase 3
STAT	signal transducers and activators of transcription
MAPK	mitogen-activated protein kinase
AKT	protein kinase B
PCR	polymerase chain reaction
ERK	extracellular signal-regulated kinase
NF-kB	B-cell nuclear factor kappa-light-chain-enhancer
IKK	IK kinase
NEMO	essential modulator-binding domain of NF-kB
HIF-1α	hypoxia inducible factor-1α
Bcl-2	B-cell leukemia/lymphoma 2
Bax	Bcl-2 associated X
EphA2	ephrin A2
cSRC	Rous sarcoma
AURKA	Aurora kinases A
AURKB	Aurora kinases B
siRNA	small interfering RNA
shRNA	short hairpin RNA
EMT	Epithelial-mesenchymal transition
HER	epidermal growth factor
Wnt	wingless-type MMTV integration site
CAR	chimeric antigen receptor
PDL1	programmed cell death 1 ligand 1
Kit	receptor tyrosine kinase
HSP	heat shock protein

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