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P-Glycoprotein and Breast Cancer Resistance Protein in Canine Inflammatory and Non-Inflammatory Grade III Mammary Carcinomas

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Abstract

P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) expression are frequently related to multidrug resistance (MDR) in neoplastic cells. Canine inflammatory and grade III non-inflammatory mammary carcinomas (IMC and non-IMC) are aggressive tumors that could benefit from chemotherapy. This study describes the immunohistochemical expression of P-gp and BCRP in 20 IMCs and 18 non-IMCs from dogs that had not received chemotherapy. Our aim was to determine if P-gp and BCRP expression were related to the “inflammatory” phenotype, to establish a basis for future studies analyzing the response to chemotherapy in dogs with highly malignant mammary cancer. Immunolabeling was primarily membranous for P-gp with a more intense labeling in emboli, and immunolabeling was membranous and cytoplasmic for BCRP. IMCs expressed P-gp in 17/20 (85%) cases while 7/18 (39%) non-IMCs expressed P-gp, with a significant higher number of IMCs versus non-IMCs (*P=0.006*). BCRP was expressed within emboli in 15/19 (79%) emboli in IMC, 12/15 (80%) primary IMC and 12/18 (67%) non-IMC, without statistically significant differences ($P>0.05$). All IMCs and 67% of non-IMCs expressed at least one of the two transporters, and 63% (12/19) of IMC and 39% (7/18) of non-IMC expressed both P-gp and BCRP. P-gp and BCRP evaluation could help select patients for chemotherapy. P-gp, expressed in a significantly higher percentage of IMC vs non-IMC, could play a specific pathogenic role in the chemoresistance of IMC.

Keywords

P-glycoprotein, breast cancer resistance protein, canine mammary carcinoma, canine inflammatory mammary carcinoma, immunohistochemistry

P-gp and BCRP are well-known adenosine triphosphate-binding cassette (ABC)-transporter efflux pumps influencing chemotherapy resistance in cancer. Due to their capacity to export a wide variety of cell substrates, including many of the currently available chemotherapeutic compounds, the pumps prevent the intracellular action of these drugs.[20] *De novo* or intrinsic multidrug resistance (MDR) occurs in a tumor when ABC-transporters such as P-gp and BCRP are expressed and functional in neoplastic cells before induction chemotherapy, while secondary or acquired expression of ABC-transporters appears after the first chemotherapy treatment.[10] P-gp and BCRP are also considered markers of the so-called side population of cancer-initiating cells. Because of this, the regulation of stem cell biology by ABC-transporters has emerged as an important new field of investigation. The complex role of P-gp and BCRP in breast cancer has been addressed in human research,[14,34] whereas data on the role of chemoresistance mediated by ABC-transporters in veterinary oncology are currently scant.,[30,68]

Canine mammary neoplasia comprises a group of tumors with heterogeneous pathological phenotypes and clinical behavior.,[55] Carcinomas of the bitch are classified into three different histological grades of malignancy according to the most recent system.[50] Histological grade III carcinomas are the rarest and have the worst prognosis with 71% of cancer-related death and/or recurrence/metastasis within a 28 months follow-up period.[50] Based on the limited clinical information available in veterinary medicine, the drugs effective in human breast cancer, such as cyclophosphamide, 5-fluorouracil, and doxorubicin, have been translated into veterinary oncology in an attempt to treat canine mammary carcinoma (CMC).[19,27,60] Treatment guidelines for malignant canine mammary gland tumors have been

proposed,[60] but the administration of a chemotherapeutic regimen to dogs with aggressive CMC has yielded conflicting results.[3,21,26,27,34,40,59,62]

Human inflammatory breast cancer (IBC) and its counterpart in the dog, canine inflammatory mammary cancer (IMC), are considered the most malignant mammary tumors.[17,41,51,52,56,57,64]IBC and IMC are clinicopathological entities diagnosed on the basis of a rapid progression of signs, dermal erythema, induration and edema of the mammary region, with the histological feature of massive embolization of neoplastic cells in superficial dermal lymphatic vessels.[52,56,64] This neoplasm bears a unique rapid-growing, angioinvasive and angiogenic phenotype, which is far from being completely understood.[17,29]

The therapeutic approach in women with IBC usually involves neoadjuvant anthracycline- and taxane-based chemotherapy, associated when feasible with ablative surgery and locoregional radiotherapy.[6,22,61,64,65] Despite recent advances in multimodality treatments, the prognosis for women with IBC is still poor, with a median disease-free survival of less than 2.5 years.[29,66] One study on medical treatment for dogs with IMC suggests a beneficial effect of nonsteroidal anti-inflammatory drugs like piroxicam as a single-agent;[37] in another study, the administration of piroxicam combined with either carboplatin, doxorubicin, capecitabine or cisplatin showed some improvement in prolonging survival time but without statistical significance.[41] New therapeutic strategies to improve survival are needed in both species. Chemotherapeutic molecules like anthracycline are substrates of many ABC-transporters, and the onset of chemoresistance to these compounds has been attributed to the expression of P-gp and BCRP in human oncology.[14,45,47] Some studies on canine mammary tumors have investigated P-gp and BCRP expression,[4,16,25,28,32,33,49,53] but the role of these chemoresistance

modulators is still incipient. A previous study designed and carried out by some of the present study's authors (ML, BB, GS, CB) evaluated the immunohistochemical expression of P-gp and BCRP in a cohort of canine mammary hyperplasia and benign and malignant mammary tumors.[35] In that study, 22/47 (47%) of hyperplastic mammary lesions expressed P-gp, and 27/45 (60%) of these lesions were BCRP positive. However, P-gp and BCRP were most commonly expressed in malignant mammary tumors with 35/46 (77%) of the cases being P-gp positive and 36/44 (82%) of the cases being BCRP-positive, while only 4/10 (40%) of adenomas expressed P-gp-positive and 2/10 (20%) expressed BCRP.[35] Other studies have reported that these chemoresistance markers are more frequently expressed in aggressive mammary tumors compared to their benign counterparts.[16,32,48,53]

Hence, the present study focused on a cohort of aggressive canine mammary tumors that included 20 IMCs and 18 grade III non-inflammatory mammary carcinomas (non-IMCs) of different histological types from dogs that had not received chemotherapy. The goal of this study was to investigate if expression of these molecules was significantly associated with the "inflammatory" phenotype and to establish a basis for future studies analyzing the response to chemotherapy of canine patients with highly malignant mammary cancer. As canine mammary tumors, especially IMC and other invasive carcinomas, have been proposed as useful spontaneous models for studying their human counterparts,[1,9,37,46,51,52,54] a better understanding of their biological behavior and chemoresistant phenotype would be helpful in both human and veterinary oncology.

Materials and Methods

Samples and histopathology

Samples were retrieved from the archive of the Veterinary Pathology Service, Veterinary Teaching Hospital, Complutense University of Madrid, Spain. According to the reports, the dogs had not received chemotherapy at the time of biopsy. Thirty-eight formalin-fixed and paraffin-embedded tissue samples of mammary gland from 38 dogs were selected based on the clinical and histopathological diagnoses of IMC and histologic grade III non-IMC. Carcinomas were graded according to the system proposed by Peña and colleagues (2013).[50] In cases of IMC, the primary mammary carcinoma was included in the study when available (n=15). All primary IMCs were histological grade III.

IMC diagnosis was based on the typical clinical presentation (with dermal erythema and edema in the ventral abdominal dermis), histologically associated with neoplastic emboli in superficial dermal lymphatic vessels. IMC emboli were detectable in each case (20/20), while the corresponding primary IMC was available in 15 cases. Hematoxylin and eosin-stained slides were histopathologically examined and when necessary the original diagnosis was updated according to the current histologic classification proposed by Goldschmidt and colleagues.[18] Only grade III carcinomas were included in the study.

Immunohistochemical expression of P-gp and BCRP

Paraffin blocks were sectioned (3 µm) and placed in a PT module (Lab Vision) containing EDTA buffer solution (pH 8.0) (Master Diagnostica, MAD-004072R/D), heated for 20 minutes at 95°C and cooled to 60°C (dewaxing and antigen retrieval). Slides were rinsed in warm tap water and placed in an automated immunostainer (Lab Vision Corporation, Fremont, CA, USA) for immunohistochemistry. Slides were incubated for 120 minutes at room temperature with the following primary antibodies: mouse monoclonal anti-P-gp/CD243 (C494, GTX23365; Manufacturer: Gene Tex

International; dilution 1:1500) and mouse monoclonal anti-BCRP (BXP-21, Millipore-MAB4146; manufacturer: Merck - dilution 1:200). Immunolabeling was detected with the peroxidase Master Polymer Plus Detection System, (Master Diagnostica, MAD-000237QK) and, subsequently, the slides were counterstained with Mayer's hematoxylin and permanently mounted with DPX mountant. External (normal canine liver) and internal (endothelium of blood and lymphatic vessels) positive controls for P-gp and BCRP were used. The cell membrane of hepatocytes (especially at the biliary canaliculi level), biliary epithelial cells and endothelial cells are normally labeled by both anti-Pgp and anti-BCRP antibodies and these served as positive controls.

Corresponding negative control slides were processed in parallel by replacing the primary antibody with non-reactive antibody. P-gp and BCRP immunolabeling was considered positive when >20% and >10% of cells were labeled for P-gp and BCRP, respectively, as suggested by previous studies.[13,35,53] A further semiquantitative evaluation of the immunostaining was performed and the positive carcinomas were assigned to 2 subgroups according to the percentage of positive cells: intermediate positivity (range of 20-50% of P-gp-positive cells; range of 10-50% of BCRP-positive cells) or high positivity (>50% of P-gp-positive cells; >50% of BCRP positive cells). One case of IMC with no available primary tumor was excluded from the evaluation of immunolabeling with anti-BCRP antibody because the internal control did not show the expected immunoreactivity with BCRP, probably due to antigen denaturation by overfixation. The selected samples included 20 IMCs and 18 non-IMCs.

Statistical analysis

P-gp and BCRP expression were compared by Fisher's exact method (GraphPad Software, La Jolla, California, USA; accessed May 2017). In IMCs, neoplastic cell

emboli were evaluated separately from the primary tumor and compared to each other. P-gp expression was evaluated in IMC emboli versus non-IMC, primary IMC versus non-IMC. BCRP expression was evaluated in IMC emboli versus non-IMC, primary IMC versus non-IMC. $P < 0.05$ was considered significant.

Results

In the 15 IMCs for which the primary carcinoma was available, histotypes of the primary IMCs included 6 micropapillary invasive carcinomas, 3 comedocarcinomas, 2 lipid-rich carcinomas, 2 solid carcinomas, 1 adenosquamous and 1 anaplastic carcinoma. Five cases of IMC did not have evidence of an associated primary mammary carcinoma; these samples where composed of dermal incisional biopsies (4 were sampled at necropsy, and 1 was submitted for histologic evaluation by a private practitioner) and histologically had massive embolization by neoplastic cells in dermal lymphatic vessels. In 8 cases of IMC, the embolized lymphatic vessels were surrounded by desmoplasia, often accompanied by severe dermal edema. Only 2 cases showed multifocal areas of intratumor colliquative necrosis, while another 2 cases had abundant inflammatory infiltrates of neutrophils, lymphocytes and plasma cells associated with the neoplasia.

Histotypes of non-IMC included 7 solid carcinomas, 5 micropapillary invasive carcinomas, and 1 each of carcinoma mixed type, tubulopapillary carcinoma, comedocarcinoma, anaplastic carcinoma, adenosquamous carcinoma, and carcinoma and malignant myoepithelioma. Eight cases of non-IMC showed multifocal areas of colliquative necrosis in more than 50% of the tumor. Examples of IMC and non-IMC histotypes are shown in Supplemental Figures S1-S4.

The diffuse membranous immunolabeling of canine normal liver, the positive control for P-gp and BCRP, is shown in Supplemental Figures S7-S8, while the negative controls are shown in Supplemental Figures S9-S10.

In tumor cells, P-gp immunolabeling was mainly membranous (Figure 1) and was particularly strong in neoplastic emboli of IMC (Figure 2). IMC emboli had cells with peculiar morphological features of severe anisokaryosis and anisocytosis. Some tumor cells stood out for their endothelial-like morphology, characterized by a rim of elongated cytoplasm containing extended eccentric nuclei encircling an empty round space and suggestive of vasculogenic mimicry. These cells were intensely immunolabeled for P-gp (Figures 3 and 4). BCRP showed membranous and cytoplasmic immunolabeling (Figures 5 and 6). Considering IMCs and non-IMCs together, P-gp was expressed in 24/38 (63%) tumors, and BCRP was expressed in 27/37 (73%) tumors. IMCs expressed P-gp in lymphatic emboli in 17/20 (85%) cases, and in 12/15 (80%) primary IMCs. Fewer non-IMCs (7/18, 39%) expressed P-gp. P-gp expression was significantly higher in emboli of IMC versus non-IMC (*P=0.006*) and in primary IMC vs non-IMC (*P=0.032*) (Supplemental Figure S5).

BCRP was expressed in 15/19 (79%) IMC emboli, 12/15 (80%) primary IMC, and 12/18 (67%) non-IMC (Supplemental Figure S6). There was no significant difference in BCRP expression between IMC and non-IMC.

Co-expression of P-gp and BCRP in the same tumor is summarized in Table1. Considering IMCs, 12/19 (63%) emboli expressed both P-gp and BCRP, and all IMCs expressed at least one of the transporters (Figures 7a and 7b). Considering non-IMCs, 7/18 (39%) expressed both P-gp and BCRP, and 12/18 (67%) expressed at least one of the transporters.

Non-IMC lacking both P-gp and BCRP expression included 2 solid, 2 micropapillary invasive, 1 adenosquamous, and 1 tubulopapillary carcinomas.

Considering the subgrouping of the positive carcinomas, IMCs showed mostly a high number of positive cells for both transporters (>50% P-gp-positive cells in 11/17 of positive cases; >50% of BCRP-positive cells in 9/15 positive cases). Of the 7 P-gp-positive primary non-IMCs, 3 contained between 20-50% of P-gp positive cells and 4 cases had >50% P-gp positive cells. Of the 12 BCRP-positive primary non-IMC cases, 3 had a between 10-50% BCRP-positive cells and 9 had >50% BCRP-positive cells.

Discussion

This is the first study to compare the expression of chemoresistance-associated markers P-gp and BCRP in canine grade III inflammatory (IMC) and comparable grade III non-inflammatory mammary carcinomas (non-IMC). These two uncommon tumor groups are biologically aggressive, especially IMC, and hence are potentially suitable for chemotherapy.

This study presents a valuable caseload as it comprises rare neoplasms. The histological malignancy of the cases selected is represented not only by the high grade (grade III) but also by numerous highly malignant histotypes: micropapillary invasive carcinomas (6/20 cases) and comedocarcinomas (3/20 cases) in the IMC group; micropapillary invasive carcinomas (5/18) and solid carcinomas (7/18) among the non-IMCs. Comedocarcinoma and solid carcinoma have been recently defined as histotypes bearing a poor prognosis.[55]

P-gp and BCRP both seem to significantly contribute tomultidrug resistance in human oncology.[14,15,34] According to human breast cancer literature, chemoresistance

associated with P-gp and BCRP expression in tumors not subjected to chemotherapy at the time of biopsy is presumably an intrinsic phenomenon and ABC-transporters' expression by neoplastic cells may lead to the failure of induction chemotherapy from the start. [10]

P-gp expression is the most widely studied mechanism of chemoresistance in breast cancer.[11,12,36,42,44,63] About 40% of breast cancers express P-gp and its expression after exposure to chemotherapeutic drugs known to be P-gp substrates is correlated with a worse response to treatment in both the adjuvant and neoadjuvant settings.[11,31,58,63]

Studies on P-gp expression in canine mammary tumors have reported P-gp protein, or *MDR-1* mRNA, and its correlation with general clinical parameters.[5,16,30,32,35,53] P-gp expression varied from 76% to 50%.[4,53] Increased P-gp expression has been found in canine mammary carcinomas compared with adenomas, and most authors agree that this finding is linked to the degree of tumor malignancy.[4,32,35,53]

The present study found that IMCs expressed P-gp in 85% of emboli and in 80% of primary carcinomas, whereas this transporter was expressed in 38% of non-IMCs. Therefore, immunohistochemistry (IHC) can detect P-gp expression in both non-IMCs and IMC. The higher percentage of P-gp positive cases (63%) in the present study compared to that of Petterino et al. could be explained by the fact that we included IMCs and grade III non-IMCs that are very aggressive, a feature related to a higher expression of chemoresistance markers.[32,53]

The chemoresistance marker BCRP has been reported to be expressed in breast cancer and has been correlated with resistance to 5-fluorouracil.[8,13,38,39,45,67] Few studies are available on BCRP expression in canine mammary tumors.[25,35,48,49] Interestingly, BCRP was reportedly expressed in all canine tumor cell lines examined

and thus it was suggested that since BCRP induces resistance to doxorubicin, treating canine mammary tumors with doxorubicin should be considered with caution.[25] Other IHC studies found a high percentage of neoplastic cells expressing BCRP and a positive correlation with the degree of canine mammary tumor malignancy.[35,48] The present study found BCRP expressed in 79% of IMC emboli, 80% of primary IMC and 67% of non-IMC. These findings are in accordance with the literature reporting that aggressive canine mammary tumors often display resistance to chemotherapeutic drugs, and that the administration of BCRP substrates should be carefully considered.

Another interesting finding in the present study is that all IMCs expressed at least one of the two pumps, while 63% of IMCs and 39% of non-IMCs coexpressed both BCRP and P-gp. This suggests these tumors are likely capable of developing resistance towards substrates of both P-gp and BCRP, bearing a more complex multidrug resistance phenotype. Expression of more than one ABC-transporter mRNA by the same tumor has been described in 56% of 103 canine mammary tumorswhere seven ABC-transporters, including P-gp and BCRP, were coexpressed. P-gp and BCRP were expressed in 92% and 100% of the tumors, respectively.[25] These findings suggest caution when substrates of P-gp and BCRP (i.e. doxorubicin, vincristine, mitoxantrone) are prescribed in the treatment of IMC and non-IMC and canine mammary gland tumors. The routine evaluation of P-gp and BCRP expression may be potentially useful to select patients for chemotherapy as some human patients appear to have a naturally more aggressive phenotype associated with high levels of baseline P-gp expression.[11,20,68]

Conclusions

The expression of P-gp and BCRP seems to be common in dogs with highly malignant non-IMC and IMC. Caution is therefore required when administering substrates of P-gp and BCRP, because these tumors may bear chemoresistance towards these drugs. Routine evaluation of MDR marker expression may serve to select patients for chemotherapy.

Future prospective studies are needed to demonstrate the association between chemoresistance and the expression of MDR markers in canine mammary cancer, and to shed more light on the complexity of its pathogenic mechanisms. This will serve to devise adequate patient-tailored chemotherapy protocols. Spontaneous canine IMC could represent a model to help understand its human counterpart, IBC, an understudied entity to date.

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References

1. Abadie J, Nguyen F, Loussouarn D, et al. Canine invasive mammary carcinomas as models of human breast cancer. Part 2: immunophenotypes and prognostic significance. *Breast Cancer Res Treat*. 2017 Oct 23;

2. Alpaugh ML, Tomlinson JS, Ye Y, Barsky SH. Relationship of Sialyl-Lewisx/a Underexpression and E-Cadherin Overexpression in the Lymphovascular Embolus of Inflammatory Breast Carcinoma. *Am J Pathol*. 2002 Aug 1;161:619–628.

3. Arenas C, Peña L, Granados-Soler JL, Pérez-Alenza MD. Adjuvant therapy for highly malignant canine mammary tumours: Cox-2 inhibitor versus chemotherapy: a case-control prospective study. *Vet Rec*. 2016 Jul 30;179:125.

4. Badowska-Kozakiewicz AM, Malicka E. Evaluation of immunohistochemical expression of P-glycoprotein in neoplasms of the mammary gland in bitches. *Pol J Vet Sci*. 2010;13:343–347.

5. Badowska-Kozakiewicz AM, Sobol M, Patera J. Expression of multidrug resistance protein P-glycoprotein in correlation with markers of hypoxia (HIF-1α, EPO, EPO-R) in invasive breast cancer with metastasis to lymph nodes. *Arch Med Sci AMS*. 2017 Oct;13:1303–1314.

6. Bourgier C, Pessoa EL, Dunant A, et al. Exclusive alternating chemotherapy and radiotherapy in nonmetastatic inflammatory breast cancer: 20 years of follow-up. *Int J Radiat Oncol Biol Phys*. 2012 Feb 1;82:690–695.

7. Bunting KD. ABC transporters as phenotypic markers and functional regulators of stem cells. *Stem Cells Dayt Ohio*. 2002;20:11–20.

8. Burger H, Foekens JA, Look MP, et al. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2003 Feb;9:827–836.

9. Caceres S, Peña L, de Andres PJ, et al. Establishment and characterization of a new cell line of canine inflammatory mammary cancer: IPC-366. *PloS One*. 2015;10:e0122277.

10. Chung HC, Rha SY, Kim JH, et al. P-glycoprotein: The intermediate end point of drug response to induction chemotherapy in locally advanced breast cancer. *Breast Cancer Res Treat Dordr*. 1997 Jan;42:65–72.

11. Clarke R, Leonessa F, Trock B. Multidrug Resistance/P-Glycoprotein and Breast Cancer: Review and Meta-Analysis. *Semin Oncol*. 2005 Dec 1;32:9–15.

12. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR,Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem Off J Histochem Soc*. 1990 Sep;38:1277–1287.

13. Diestra JE, Scheffer GL, Català I, et al. Frequent expression of the multi-drug resistance-associated protein BCRP/MXR/ABCP/ABCG2 in human tumours detected by the BXP-21 monoclonal antibody in paraffin-embedded material. *J Pathol*. 2002 Oct;198:213–219.

14. Faneyte IF, Kristel PMP, Maliepaard M, et al. Expression of the breast cancer resistance protein in breast cancer. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2002 Apr;8:1068–1074.

15. Gameiro M, Silva R, Rocha-Pereira C, et al. Cellular Models and In Vitro Assays for the Screening of modulators of P-gp, MRP1 and BCRP. *Molecules*. 2017 Apr 8;22:600.

16. Ginn PE. Immunohistochemical detection of P-glycoprotein in formalin-fixed and paraffin-embedded normal and neoplastic canine tissues. *Vet Pathol*. 1996 Sep;33:533–541.

17. Giordano SH, Hortobagyi GN. Inflammatory breast cancer: clinical progress and the main problems that must be addressed. *Breast Cancer Res BCR*. 2003;5:284–288.

18. Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and grading of canine mammary tumors. *Vet Pathol*. 2011 Jan;48:117–131.

19. Goldschmidt MH, Peña L, Zappulli V. Tumors of the Mammary Gland. In: *Donald J. Meuten Tumors in Domestic Animals*. Ames, Iowa: John Wiley & Sons Inc.; 2017:722–765.

20. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP–dependent transporters. *Nat Rev Cancer*. 2002 Jan 1;2:nrc706.

21. Gustafson KU, Page RL. Cancer Chemotherapy. In: *Withrow & MacEwen's Small Animal Clinical Oncology*. St. Louis, Missouri: Elsevier Saunders; 2013:156–179.

22. Harris EER, Schultz D, Bertsch H, Fox K, Glick J, Solin LJ. Ten-year outcome after combined modality therapy for inflammatory breast cancer. *Int J Radiat Oncol Biol Phys*. 2003 Apr 1;55:1200–1208.

23. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci U S A*. 2004 Sep 28;101:14228–14233.

24. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer*. 2013 Oct;13:714–726.

25. Honscha KU, Schirmer A, Reischauer A, Schoon H-A, Einspanier A, Gäbel G. Expression of ABC-transport proteins in canine mammary cancer: consequences for chemotherapy. *Reprod Domest Anim Zuchthyg*. 2009 Jul;44 Suppl 2:218–223.

26. Karayannopoulou M, Kaldrymidou E, Constantinidis TC, Dessiris A. Adjuvant post-operative chemotherapy in bitches with mammary cancer. *J Vet Med A Physiol Pathol Clin Med*. 2001 Mar;48:85–96.

27. Karayannopoulou M, Lafioniatis S. Recent advances on canine mammary cancer chemotherapy: A review of studies from 2000 to date. *Rev Médecine Vét*. 2016;167:191–200.

28. Kim N-H, Hwang Y-H, Im K-S, et al. P-glycoprotein expression in canine mammary gland tumours related with myoepithelial cells. *Res Vet Sci*. 2012 Dec;93:1346–1352.

29. Kleer CG, van Golen KL, Merajver SD. Molecular biology of breast cancer metastasis. Inflammatory breast cancer: clinical syndrome and molecular determinants. *Breast Cancer Res BCR*. 2000;2:423–429.

30. Klopfleisch R, Kohn B, Gruber AD. Mechanisms of tumour resistance against chemotherapeutic agents in veterinary oncology. *Vet J Lond Engl 1997*. 2016 Jan;207:63–72.

31. Koh EH, Chung HC, Lee KB, et al. The value of immunohistochemical detection of P-glycoprotein in breast cancer before and after induction chemotherapy. *Yonsei Med J*. 1992 Jun;33:137–142.

32. Koltai Z, Vajdovich P. Expression of multidrug resistance membrane transporter (Pgp) and p53 protein in canine mammary tumours. *Acta Vet Hung*. 2014 Jun;62:194–204.

33. Król M, Pawłowski KM, Majchrzak K, Mucha J, Motyl T. Canine mammary carcinoma cell line are resistant to chemosensitizers: verapamil and cyclosporin A. *Pol J Vet Sci*. 2014;17:9–17.

34. Leonessa F, Clarke R. ATP binding cassette transporters and drug resistance in breast cancer. *Endocr Relat Cancer*. 2003 Mar;10:43–73.

35. Levi M, Brunetti B, Sarli G, Benazzi C. Immunohistochemical Expression of P-glycoprotein and Breast Cancer Resistance Protein in Canine Mammary Hyperplasia, Neoplasia and Supporting Stroma. *J Comp Pathol*. 2016 Nov 1;155:277–285.

36. Linn SC, Giaccone G, van Diest PJ, et al. Prognostic relevance of P-glycoprotein expression in breast cancer. *Ann Oncol Off J Eur Soc Med Oncol*. 1995 Sep;6:679–685.

37. de M Souza CH, Toledo-Piza E, Amorin R, Barboza A, Tobias KM. Inflammatory mammary carcinoma in 12 dogs: clinical features, cyclooxygenase-2 expression, and response to piroxicam treatment. *Can Vet J Rev Veterinaire Can*. 2009 May;50:506–510.

38. Mao Q, Unadkat JD. Role of the breast cancer resistance protein (BCRP/ABCG2) in drug transport--an update. *AAPS J*. 2015 Jan;17:65–82.

39. Mao Q, Unadkat JD. Role of the breast cancer resistance protein (ABCG2) in drug transport. *AAPS J*. 2005 May 11;7:E118-133.

40. Marconato L, Lorenzo RM, Abramo F, Ratto A, Zini E. Adjuvant gemcitabine after surgical removal of aggressive malignant mammary tumours in dogs. *Vet Comp Oncol*. 2008 Jun;6:90–101.

41. Marconato L, Romanelli G, Stefanello D, et al. Prognostic factors for dogs with mammary inflammatory carcinoma: 43 cases (2003-2008). *J Am Vet Med Assoc*. 2009 Oct 15;235:967–972.

42. Merkel DE, Fuqua SA, McGuire WL. P-glycoprotein in breast cancer. *Cancer Treat Res*. 1989;48:97–105.

43. Moitra K. Overcoming Multidrug Resistance in Cancer Stem Cells. *BioMed Res Int*. 2015 [cited 2017 Dec 2];2015.

44. Murray S, Briasoulis E, Linardou H, Bafaloukos D, Papadimitriou C. Taxane resistance in breast cancer: mechanisms, predictive biomarkers and circumvention strategies. *Cancer Treat Rev*. 2012 Nov;38:890–903.

45. Nakanishi T, Ross DD. Breast cancer resistance protein (BCRP/ABCG2): its role in multidrug resistance and regulation of its gene expression. *Chin J Cancer*. 2012 Feb;31:73–99.

46. Nguyen F, Peña L, Ibisch C, et al. Canine invasive mammary carcinomas as models of human breast cancer. Part 1: natural history and prognostic factors. *Breast Cancer Res Treat*. 2017 Oct 30;

47. Nieth C, Lage H. Induction of the ABC-transporters Mdr1/P-gp (Abcb1), mrpl (Abcc1), and bcrp (Abcg2) during establishment of

multidrug resistance following exposure to mitoxantrone. *J Chemother Florence Italy*. 2005 Apr;17:215–223.

48. Nowak M, Madej JA, Dziegiel P. Expression of Breast Cancer Resistance Protein (BCRP-1) in canine mammary adenocarcinomas and adenomas. *Vivo Athens Greece*. 2009 Oct;23:705–709.

49. Pawłowski KM, Mucha J, Majchrzak K, Motyl T, Król M. Expression and role of PGP, BCRP, MRP1 and MRP3 in multidrug resistance of canine mammary cancer cells. *BMC Vet Res*. 2013 Jun 17;9:119.

50. Peña L, De Andrés PJ, Clemente M, Cuesta P, Pérez-Alenza MD. Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. *Vet Pathol*. 2013 Jan;50:94–105.

51. Peña L, Perez-Alenza MD, Rodriguez-Bertos A, Nieto A. Canine inflammatory mammary carcinoma: histopathology, immunohistochemistry and clinical implications of 21 cases. *Breast Cancer Res Treat*. 2003 Mar;78:141–148.

52. Pérez Alenza MD, Tabanera E, Peña L. Inflammatory mammary carcinoma in dogs: 33 cases (1995-1999). *J Am Vet Med Assoc*. 2001 Oct 15;219:1110–1114.

53. Petterino C, Rossetti E, Bertoncello D, et al. Immunohistochemical detection of P-glycoprotein (clone C494) in canine mammary gland tumours. *J Vet Med A Physiol Pathol Clin Med*. 2006 May;53:174–178.

54. Raposo TP, Arias-Pulido H, Chaher N, et al. Comparative aspects of canine and human inflammatory breast cancer. *Semin Oncol*. 2017 Aug 1;44:288–300.

55. Rasotto R, Berlato D, Goldschmidt MH, Zappulli V. Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. *Vet Pathol*. 2017 Jul 1;54:571–578.

56. Robertson FM, Bondy M, Yang W, et al. Inflammatory breast cancer: the disease, the biology, the treatment. *CA Cancer J Clin*. 2010 Dec;60:351–375.

57. Rueth NM, Lin HY, Bedrosian I, et al. Underuse of Trimodality Treatment Affects Survival for Patients With Inflammatory Breast Cancer: An Analysis of Treatment and Survival Trends From the National Cancer Database. *J Clin Oncol*. 2014 Jul 1;32:2018–2024.

58. Sanfilippo O, Ronchi E, De Marco C, Di Fronzo G, Silvestrini R. Expression of P-glycoprotein in breast cancer tissue and in vitro

resistance to doxorubicin and vincristine. *Eur J Cancer Clin Oncol*. 1991 Jan 1;27:155–158.

59. Simon D, Schoenrock D, Baumgärtner W, Nolte I. Postoperative adjuvant treatment of invasive malignant mammary gland tumors in dogs with doxorubicin and docetaxel. *J Vet Intern Med*. 2006 Oct;20:1184–1190.

60. Sorenmo K. Canine mammary gland tumors. *Vet Clin North Am Small Anim Pract*. 2003 May;33:573–596.

61. Takiar V, Akay CL, Stauder MC, et al. Predictors of durable no evidence of disease status in de novo metastatic inflammatory breast cancer patients treated with neoadjuvant chemotherapy and post-mastectomy radiation. *SpringerPlus*. 2014;3:166.

62. Tran CM, Moore AS, Frimberger AE. Surgical treatment of mammary carcinomas in dogs with or without postoperative chemotherapy. *Vet Comp Oncol*. 2016 Sep;14:252–262.

63. Trock BJ, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. *J Natl Cancer Inst*. 1997 Jul 2;89:917–931.

64. van Uden DJP, van Laarhoven HWM, Westenberg AH, de Wilt JHW, Blanken-Peeters CFJM. Inflammatory breast cancer: an overview. *Crit Rev Oncol Hematol*. 2015 Feb;93:116–126.

65. Van der Auwera I, Van Laere SJ, Van den Eynden GG, et al. Increased angiogenesis and lymphangiogenesis in inflammatory versus noninflammatory breast cancer by real-time reverse transcriptase-PCR gene expression quantification. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2004 Dec 1;10:7965–7971.

66. Woodward WA. Inflammatory breast cancer: unique biological and therapeutic considerations. *Lancet Oncol*. 2015 Nov;16:e568-576.

67. Yuan J-H, Cheng J-Q, Jiang L-Y, et al. Breast cancer resistance protein expression and 5-fluorouracil resistance. *Biomed Environ Sci BES*. 2008 Aug;21:290–295.

68. Zandvliet M, Teske E. Mechanisms of Drug Resistance in Veterinary Oncology- A Review with an Emphasis on Canine Lymphoma. *Vet Sci*. 2015 Aug 12;2:150–184.

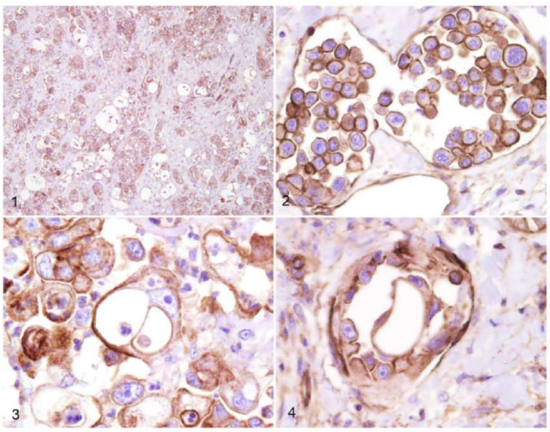

Figure 1-4. Canine inflammatory mammary cancer (IMC), mammary gland, dog. IHC for P-gp. Figure 1. Intense membranous expression of P-gp in neoplastic cells of a primary IMC. Figure 2. Emboli of IMC with intense membranous expression of P-gp. The endothelium of lymphatic vessel, considered a positive internal control, is positive. Figures 3-4. IMC, mammary gland, dog. Intense immunolabeling with P-gp of neoplastic cells with morphological features suggestive of vasculogenic mimicry.

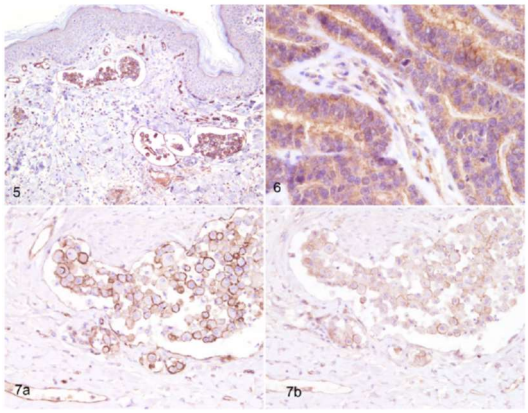

Figure 5-7. Mammary gland, dog. IHC for BCRP and P-gp. Figure 5. IMC. Intense membranous expression of BCRP in emboli of IMC in superficial dermal lymphatic vessels. The endothelium of lymphatic vessel, considered a positive internal control, is positive. Figure 6. Canine non-IMC histologic grade III mammary carcinoma, tubulopapillary histotype. Intense cytoplasmic and membranous expression of BCRP in neoplastic cells. Figure 7. Embolus of mammary inflammatory carcinoma. Neoplastic cells, in the same area, express both P-gp (Figure 7a) and BCRP (Figure 7b), with strong P-gp and mderate BCRP membranous immunolabeling and minimal cytoplasmic immunolabeling for both transporters.

Supplemental material figure legends

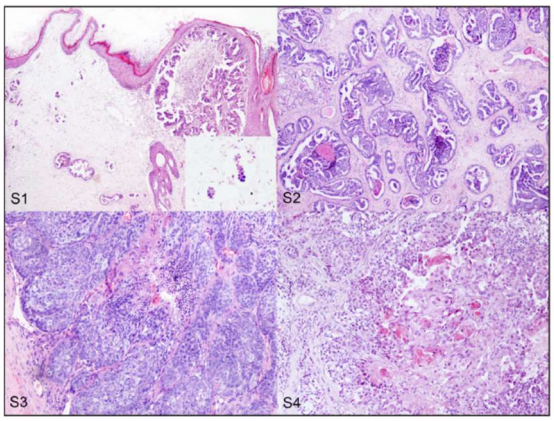

Figure S1-2. Canine inflammatory mammary cancer (IMC), mammary gland, dog. Figure S1. Massive embolization of neoplastic cells in superficial dermal lymphatic vessels (inset at higher magnification), associated with interstitial dermal edema and moderate inflammatory infiltrate. HE. Figure S2. Primary IMC of micropapillary invasive histotype. HE. **Figure S3-4.** Canine non-inflammatory histologic grade III mammary carcinoma, mammary gland, dog. Figure S3. Solid histotype. HE. Figure S4. Adenosquamous histotype. HE.

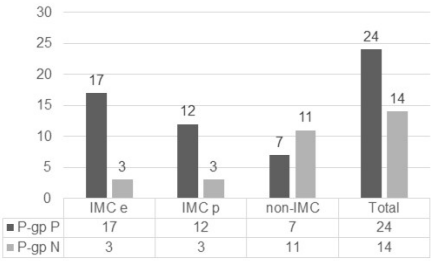

Figure S5. P-gp expression in neoplastic emboli (emboli IMC) and primary (primary IMC) tumor of canine inflammatory mammary cancer (IMC), and canine non-inflammatory histologic grade III mammary carcinoma (non-IMC). Emboli of IMC expressed P-gp in 85% of the cases (17/20), and primary IMC expressed P-gp in 80% of the primary IMCs (12/15). Fewer cases of non-IMCs expressed P-gp (7/18, 38.89%).

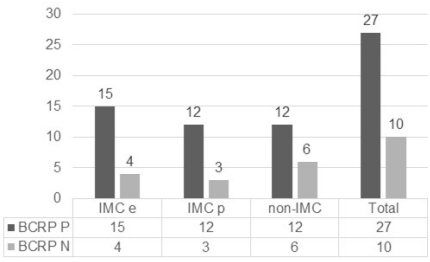

Figure S6. BCRP expression in neoplastic emboli (emboli IMCe) and primary (primary IMCp) tumor of canine inflammatory mammary cancer (IMC), and canine non-inflammatory histologic grade III mammary carcinoma (non-IMC). BCRP was

expressed in 78.95% of IMC emboli (15/19), 80% of primary IMC (12/15) and 66.67% of non-IMC (12/18).

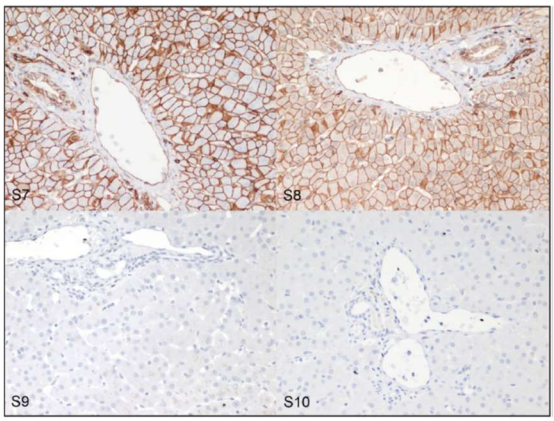

Figure S7. Liver, dog. IHC for P-gp. Intense P-gp immunolabelling of the plasma membranes of the hepatocytes, biliary epithelium and endothelial cells of hematic and lymphatic vessels in the positive control . **Figure S8**. Liver, dog. IHC for BCRP. Intense BCRP immunolabelling of the plasma membranes of the hepatocytes, biliary epithelium and endothelial cells of hematic and lymphatic vessels in the positive control. **Figure S9.** Liver, dog. IHC. P-gp expression in the negative control. Positive immunolabeling is absent. **Figure S10.** Liver, dog. IHC.BCRP expression in the negative control. Positive immunolabeling is absent.

Table 1. Expression of both P-gp and BCRP in canine inflammatory and grade III non-inflammatory mammary carcinomas.

	Canine Inflammatory Mammary Cancer (n=19)§	Canine grade III non-inflammatory Mammary Carcinomas (n=18)
P-gp and BCRP positive	63% (12/19)	39% (7/18)
P-gp positive; BCRP negative	21% (4/19)*	0% (0/18)
P-gp negative; BCRP positive	16% (3/19)	28% (5/18)
P-gp and/or BCRP positive	100% (19/19)	67% (12/18)
P-gp and BCRP negative	0% (0/19)	33% (6/18)

Abbreviations: P-gp, P-glycoprotein; BCRP, Breast Cancer Resistance Protein.

§: in emboli of IMC

*: one case excluded because BCRP was not reactive