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比較使用必諾賓與艾黴素治療貓乳癌之療效與毒性

Comparison of Efficacy and Toxicity of Vinorelbine and Doxorubicin in Feline Mammary Carcinoma

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本論文係 董育廷君（學號 R03643009）在國立臺灣大學臨床動物醫學研究所完成之碩士學位論文，於民國 107 年 1 月 23 日承下列考試委員審查通過及口試及格，特此證明

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中文摘要

乳癌為貓癌症中第三常見的惡性腫瘤，若罹患乳癌的貓伴隨有高惡性程度腫瘤、腫瘤直徑超過三公分、腫瘤已侵犯淋巴管、血管、淋巴結或遠端轉移，單純的手術切除往往無法達到有效的腫瘤控制，而需要全身性的化學治療來輔助治療的效果，艾黴素為現今最常使用於乳癌的化療藥物之一，然而目前尚缺乏前瞻性且隨機分組的研究來證實艾黴素能夠有效增長存活時間，此外許多文獻都發現以艾黴素治療貓乳癌常伴隨有消化道毒性與累積性的腎臟毒性。必諾賓為一種半合成的長春花生物鹼類化療藥，此藥也被用在治療人類乳房癌中，且消化道毒性大多輕微。因此本研究的目的是要比較必諾賓與艾黴素在貓乳癌治療的有效性與毒性，同時分析可能影響預後的因子。

本研究以前瞻性的方式收集了於西元 2016 年六月起在國立臺灣大學生物資源暨農學院附設動物醫院透過組織病理學或細胞學確診為惡性乳癌並且使用必諾賓為第一線化療的患貓，記錄其病歷資料與治療過程及其反應，另外以回溯性的方式收集了在國立臺灣大學生物資源暨農學院附設動物醫院中，西元 2014 年至 2017 年罹患乳癌、並以艾黴素作為其第一線化療的患貓其病歷資料作為艾黴素組，分析兩組間腫瘤與病患特徵分佈、治療效果、與毒性是否有統計顯著性差異，同時對所有病患評估可能的預後因子的影響。

研究最終收集了 26 個案例，其中 7 隻患貓為必諾賓組，19 隻為艾黴素組，兩組在腫瘤特徵與臨床分期分佈上無顯著差異，但兩組都將近有 60％的患貓為臨床分期第四期的病患，在效果的部分，必諾賓組的反應率為 75%，而艾黴素組為 18%，生物反應率的定義在本研究為病患在接受化療時，曾經因為化療藥物而導致腫塊消退、維持穩定或保持無巨觀腫塊的比例，必諾賓組的生物反應率為 100%，艾黴素組則為 42%，兩組在生物反應率有顯著差異。必諾賓組的中位腫瘤惡化時間（Time to progression）為 115 天，而艾黴素組為 102 天，兩組無顯著差異（P =0.949）；而在中位存活時間的分析當中，必諾賓組為 352 天，艾黴素組為 284 天，兩組亦無顯著差異（P =0.948）。然而在毒性的部分，必諾賓組的嘔吐發生率顯著地低於艾黴素組（P =0.004），且食慾不振的發生率亦顯著小於艾黴素組（P <0.001）；

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白血球低下的骨髓毒性則顯著的在必諾賓組較高（*P* <0.001），但沒有發生敗血症或與白血球低下相關的任何臨床症狀。預後因子分析的結果顯示，單變數與多變數分析中，會造成腫瘤惡化時間縮短的因子為已絕育的母貓罹患乳癌與對於化療無生物反應之患貓（Non-biological responders）；對於化療無生物反應在單變數與多變數分析中均顯示有較短的存活時間，而腫塊出現潰瘍僅在多變數分析中顯示會縮短存活時間。

本研究的結果指出，必諾賓與艾黴素對於貓癌的治療效果相似，雖然使用必諾賓較可能出現白血球低下的副作用，但在本研究中白血球低下並沒有導致任何相關臨床症狀，且必諾賓有更低的消化道毒性，因此必諾賓或許更適合用在貓乳癌的化療當中，此外，罹患乳腺腫瘤時絕育的狀態、腫塊是否潰瘍與對化療是否有生物反應則為本研究的預後因子。

關鍵字：貓乳癌、必諾賓、艾黴素

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ABSTRACT

Feline mammary carcinomas (FMC) are the third most common cancer in cats. For those cats with large primary tumors, histological high grade tumors, evidence of lymphatic or vascular invasion, lymph node or distant metastasis, surgery alone is rarely effective and curative. Adjuvant chemotherapy is often recommended to improve outcome. Doxorubicin (DOX) is the most commonly used chemotherapy agent for mammary carcinoma. However, it is still lack solid evidence of survival benefit from adjuvant doxorubicin therapy. In addition, doxorubicin can be nephrotoxic to cats and often cause gastrointestinal disturbance. Vinorelbine (VRL) is a semisynthetic derivative of vinca alkaloids and can widely distribute in most tissues, especially in lung. In human, vinorelbine can be also used to treat breast cancer. Therefore, the purpose of this study is to compare the efficacy and toxicity of vinorelbine and doxorubicin in FMC. Possible prognostic factors for FMC were also investigated.

Cats diagnosed with FMC histologically or cytologically and had been received doxorubicin or vinorelbine as their first-line chemotherapy at National Taiwan University Veterinary Hospital (NTUVH) animal cancer treatment center were enrolled into this study and the patient were divided into VRL group or DOX group. Cats in VRL group were enrolled prospectively since June, 2016 and cats in DOX group were collected retrospectively from 2014 to 2017 at NTUVH animal cancer treatment center. Total of twenty-six cats were divided into the two groups based on their initial treatment. Seven cats received vinorelbine as their first-line chemotherapeutic agent and were assigned to the VRL group; nineteen cats receiving doxorubicin as their first-line chemotherapeutic agent and assigned to the DOX group. No significance in distribution of tumor

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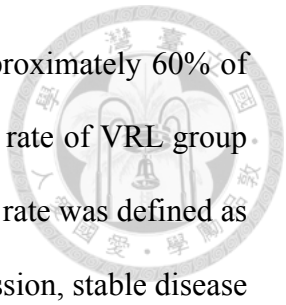

characteristics distribution and clinical stage in the two groups. Approximately 60% of patients were classified as clinical stage 4 in both groups. Response rate of VRL group and DOX group was 75% and 18% respectively. Biological response rate was defined as the percentage of cats had been experienced complete or partial remission, stable disease and Non-CR/non-PD in this study. Biological response rate was 100% for VRL group and 42% for DOX group, which was statistical significant. Median time to progression was 115 days for VRL group and 102 days for DOX group, which was not statistically significant (P =0.949). Median survival time was 352 days for VRL group and 284 days for DOX group, but no significant difference was noted (P =0.948). However, the incidence of vomiting and anorexia were both significantly higher in DOX group (P =0.004 and <0.001, respectively). Although the incidence of neutropenia was significantly higher in VRL group, no evidence of sepsis or clinical signs related to neutropenia was observed. As for the results of prognostic factor analysis, intact females and biological responders were both significantly associated with longer time to progression in univariate and multivariate analysis. Ulceration of tumor was correlate to worse survival only in multivariate analysis. Biological responders had significantly longer survival in both univariate and multivariate analysis.

In conclusion, the efficacy of vinorelbine was similar to doxorubicin but much less gastrointestinal toxicities observed in cats treated with vinorelbine. Therefore, vinorelbine may be more appropriate to use as adjuvant chemotherapy in FMC.

Keywords: Feline mammary carcinoma, vinorelbine, doxorubicin

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Chapter 1 Literature review

1.1 Feline mammary gland tumor

Feline mammary gland tumor is a common neoplastic disease in feline and mostly occurs in female cats. Based on a previous investigation in California, the annual incidence rate in female cats was 25.4/100,000 [3], which is much lower than female dogs (198/100,000). Unlike dogs, approximate 90% of mammary gland masses are malignant [6]. Most malignant mammary gland tumors in cats are carcinomas; sarcomas are rare, which is also different from canine MGTs [38]. Most feline mammary carcinomas occur at the median age of 10 to 12 years, older neutered female cats or intact female cats are at higher risk [38]. Siamese cats appear overrepresented, but this breed cats are also high risk of developing other neoplasms [38].

1.1.1 Benign mammary gland masses

Benign mammary gland masses are rare. In one study, all cats diagnosed with benign mammary masses were all intact females and younger than 10 years old [12]. Fibroadenoma, other adenomas, duct papilloma, cyst, lobular hyperplasia, and fibroadenomatous hyperplasia were all benign lesions previously reported. Ovariohysterectomy (OHE) is a curative treatment in cats with fibroadenomatous hyperplasia; as for other benign lesions, complete surgical removal can be also curative and is important to prevent those lesions transforming to malignant [6].

1.1.2 Feline mammary carcinoma (FMC)

The etiology of developing FMC remains uncertain. Since intact and older-neutered females are overrepresented, sex hormone may play an important role of developing FMC.

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It is well established that OHE at early age can reduce the risk of developing FMC. Ovariohysterectomy before age of 6 months, between 7 and 12 months, or 1 and 2 years can reduce the risk of developing FMC approximately 91%, 86%, and 11% respectively [33]. However, this protection effect was not seen in cats underwent OHE after 2 years old.

Feline mammary carcinomas can be further classified as adenocarcinoma, tubular carcinoma, cystic papillary carcinoma, cribriform carcinoma, micropapillary invasive carcinoma, comedocarcinoma, squamous cell carcinoma, mucinous carcinoma, and lipid-rich carcinoma [28]. Adenocarcinoma, tubular carcinoma, or a combination of tubular, papillary, and solid carcinoma are most common subtypes [38]. Feline mammary carcinomas are generally aggressive. They can grow rapidly and have ability to invade lymphatic and vascular vessels in order to metastasize. Regional lymph node, lung, pleura and liver are organs that commonly being metastasized [10].

Cats typically presented with palpable subcutaneous masses at mammary gland region; single, multiple or even bilateral mammary glands may be involved. Masses may be ulcerative and with discharge in some cases. For early stage patients, there are usually no other specific clinical signs. Affected lymph node can be in normal size when it contains few tumor cells, but it can become large and fixed in late stage. Edema of limbs may be found when lots tumor cells metastasize to regional lymph nodes or tumor emboli in vessels obstruct fluid return. Pain reaction can be observed in cats with ulcerative or inflamed tumors and cats with bone metastasis. Dyspnea, panting, paradoxical breathing, and pleural effusion are common in cats with pulmonary metastasis. Non-specific signs such as lethargy, poor appetite and weight loss are usually noted in advanced stage patients.

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1.2 Treatment of mammary carcinoma

1.2.1 Surgery

Surgery is an important treatment in FMC. Aggressive and radical surgical procedure can provide favorable outcome for early stage patients. Unilateral chain mastectomy with removal of draining lymph nodes for cats presented with ipsilateral tumors and bilateral chain mastectomy with removal lymph nodes for cats presented with contralateral tumors are usually recommended [38]. Marginal excision of affected mammary glands or lumpectomy usually results in incomplete excision, and those procedures are therefore only recommended for advanced stage patients to relieve discomfort. In a retrospective study, 100 cats with FMC were reviewed [20], and the disease-free interval was significantly (P <0.01) longer for cats underwent radical surgery than cats with conservative surgery. The disease-free interval and overall survival time was 372 days and 1,406 days respectively for cats underwent surgery alone in another study [26].

1.2.2 Systemic therapy

Systemic treatment is usually recommended in FMC, especially for cats with high risk of local recurrence and metastasis. Tamoxifen, a selective estrogen receptor modulator, is commonly used as a first-line adjuvant therapy in human with hormone-receptor-positive breast cancer [29]. However, this agent used in feline is unlikely as effective as in human, because expression of estrogen receptors in FMC are relative low [38].

Chemotherapy is recommended in human with hormone-receptor-negative breast cancer, failure of endocrine therapy or presence of visceral metastasis [29].

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Anthracyclines and taxanes are their first choice, and other agents such as cisplatin, vinorelbine, alkylating agents and anti-metabolites were also documented to have activity against breast cancer in human. Similarly, doxorubicin-based chemotherapy is the most commonly used scheme in small animals. However, due to lack of a prospective, randomized controlled study in veterinary medicine, outcome of the cats treated with adjuvant chemotherapy varied in different retrospective studies. Fourteen cats with advanced stage mammary gland adenocarcinoma were treated with doxorubicin and cyclophosphamide [25]. Overall response rate was 50% and all responders experienced partial remission. The mean and median survival times of the 14 cats were 142 days and 90 days, respectively, and responders trended to have longer survival times (median survival time was 150 days and 75 days for responders and non-responders respectively), but there was no control group in the study. Another single-arm, multi-institutional retrospective study evaluated the outcome of 67 cats treated with doxorubicin alone [31]. The median disease-free interval was 255 days and the median survival time was 448 days in the study. A similar result was reported in another study; twenty-three cats treated with surgery, doxorubicin-based chemotherapy and meloxicam (a cyclooxygenase-2 inhibitor) and the median disease-free interval and median survival time was 269 days and 460 days respectively [1]. Unfortunately, those studies were all lack of control group to compare the efficacy of chemotherapy. In a non-randomized, retrospective study by McNeill et al [26], 73 cats were enrolled to compare the survival time between cats underwent surgery alone and cats underwent surgery and doxorubicin-based adjuvant chemotherapy. Although the rate of local recurrence and distant metastasis was slightly higher in surgery alone group, the difference was not significant. In addition, the median survival time between two groups was also non-significant (1,406 days for surgery alone group and 848 days for surgery combined chemotherapy group). Nevertheless, cats

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received radical unilateral surgery and followed with chemotherapy had significant longer survival than cats underwent radical unilateral surgery alone in this study. A two-arm, retrospective study compared the efficacy of carboplatin in cats with advanced stage mammary carcinoma [2]. In that study, 9 cats underwent unilateral radical mastectomy and 7 cats received surgery and followed with 200 mg/m^2 carboplatin every 3 weeks. The median survival time for cats treated with adjuvant chemotherapy was 428 days, longer than 387 days for cats treated with surgery alone, but the difference was not significant. Although the benefit of adjuvant chemotherapy remains controversial in veterinary medicine due to lack of solid evidence from prospective randomized studies, it is still recommended to prescribe adjuvant chemotherapy for cats with larger tumors (diameter of tumor >3 cm), evidence of lymphatic/blood vessel invasion and lymph node metastasis.

1.3 Vinorelbine

Vinorelbine, a semi-synthetic vinca alkaloid, was first produced in 1979. Same as most vinca alkaloid, vinorelbine can cause cell death by inhibiting the polymerization of tubulin dimers into microtubules, and prevents cell division, mitotic arrest, and ultimately cell death [30]. Lipophilicity of vinorelbine is higher than other vinca alkaloids; hence, this drug distributed widely with high concentrations in all tissues, except brain. Besides, the concentration of this drug in lung tissue was 300-fold higher than in plasma [18]. Vinorelbine is metabolized by liver and eliminated mainly via biliary system and minor by renal. In human medicine, vinorelbine is mostly used in non-small cell lung cancer, and can be also used in non-Hodgkin lymphoma and breast cancer. In a phase II study in human [5], 157 chemotherapy-naïve patients with advanced or metastatic breast cancer were administer with a weekly 30 mg/m^2 vinorelbine; the overall response rate was 41%

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(complete response, 7%; partial response, 34%) and 30% of patients had stable disease. Neutropenia was the most common toxicity and few episodes of nausea or vomiting were observed in that study. Vinorelbine was found to be active as a single agent (overall response rate 25% to 45%), even in patients heavily pretreated with anthracyclines and taxanes [14], [35]. There were only few studies of vinorelbine in veterinary medicine. A dose-determining study by Poirier et al [35], a starting dosage of 15 mg/m^2 for dogs was recommended and neutropenia was the dose-limiting toxicity; overall response rate was 12.5% in various and spontaneous tumor-bearing dogs. Another phase II study evaluated the efficacy of vinorelbine in dogs with cutaneous mast cell tumors [8]; overall response rate was 13% and a high prevalence of neutropenia was found in this study. Kaye et al used this drug as a rescue agent for dogs with primary urinary bladder carcinoma [15]. Although the overall response rate was 14%, fifty-seven percent of patients experienced stable disease and 78% of patients had subjective improvement in clinical signs. Adverse events were mild and tolerated in that study. There was one but only one study used vinorelbine in feline. In that phase I study for feline [34], a starting dosage of 11.5 mg/m^2 was recommended. Similarly, neutropenia was the mostly observed adverse events for cats.

1.4 Doxorubicin

Doxorubicin is one of antitumor antibiotics which is widely used in both human and small animals. Doxorubicin can cause cell death by multimodal mechanism [9]. Doxorubicin can interact with deoxyribonucleic acid (DNA) by intercalation and alkylation. It also has the ability to inhibit the function of RNA and DNA polymerases and topoisomerase II. Generation of reactive oxygen, perturbation of cellular Ca^{2+}

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homeostasis, and inhibition of thioredoxin reductase were also reported to cause cellular toxicity. Doxorubicin is mainly metabolized by liver and eliminated via kidneys and biliary system. This drug is often used in lymphoma, soft tissue sarcoma and mammary gland tumors. Common dosing regimen in dogs is 30 mg/m^2 intravenous infusion over 30 minutes, but 1 mg/kg or 20 mg/m^2 is often substituted for 30 mg/m^2 in dogs less than 15 kg and in all cats. This drug can only be delivered intravenously; if this drug is delivered external to the vein, it will lead to severe tissue damage. Gastrointestinal toxicity and myelosuppression are most common toxicities. Some dogs may experience hypersensitivity to this drug. It is well established that there is cumulative dose-related cardiotoxicity and nephrotoxicity in small animals [9], [32]. Comparison of basic characteristics of vinorelbine and doxorubicin was summarized in Table 1.

1.5 Prognostic factors

Several prognostic factors had been reported. However, most studies were retrospective and non-randomized; thus, some factors may be biased.

Tumor size is the most important prognostic factor. Cats with tumor smaller than 2 cm had an average 4.5 years of survival time, and in contrast, cats with tumor larger than 3 cm had a median survival time of only 6 months [20]. This correlation was also observed in many other reports [27], [31], [40]. Histopathological grade was also reported to have a strong correlation with survival time in many studies [27], [37], [40]. Median survival time for cats with grade 3 mammary carcinoma was only 5 to 8 months. In contrast, cats with grade 1 mammary carcinoma had a median survival about 27 to 36 months, and the median survival time was range 12 to 14 months for grade 2 tumors. Lymph node status is another factor that impacts survival. Median survival time was 9 months for cats with

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lymph node metastasis and 16 months for cats without lymph node metastasis [27]. The clinical stage is classified based on tumor size, lymph node status and whether presence of distant metastasis. Thus, it is not surprising that clinical stage at presentation also associated with survival. Median survival time was 29, 12.5, 9 and 1 months for cats with clinical stage of 1, 2, 3, 4, respectively [13]. Cats with clinical stage of 1, 2, 3 had median survival time of 18, 15, 10 months respectively, in another study [27].

Other possible prognostic factors included histopathological subtype, with or without lymphatic and vascular invasion, presence of ulcerative tumor and type of surgical procedure. Histopathological complex carcinoma presents with neoplastic epithelial and myoepithelial cells, and most of this type carcinomas were associated with grade 1 tumors [37]. Cats with cribriform carcinomas had significantly shorter survival time [27]. Presence of lymphatic and invasion was a negative prognostic factor in several studies [27], [31], [37]. Ulcerative tumors were reported to be associated with high histopathological grade [37]. Marginal excision often leads to incomplete margin and early recurrence. Cats underwent radical unilateral surgery had significantly longer disease-free interval [13], [20]. However, this result may be confounding with tumor size and clinical stage.

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Chapter 2 Introduction

Feline mammary carcinomas (FMC) are the most common mammary tumors in cats and usually with aggressive tumor behavior [6]. Surgical excision is the treatment of choice for FMC. Aggressive surgery such as unilateral or even bilateral mastectomy is often needed to obtain complete excision and results in long- term survival for early stage cats. In contrast, for those cats with large primary tumors, histological high grade tumors, evidence of lymphatic or vascular invasion, lymph node or distant metastasis, surgery alone is rarely effective and curative [38]. Adjuvant chemotherapy is often recommended to improve outcome. Doxorubicin is the most commonly used chemotherapy agent for malignant mammary carcinoma in small animals [6], [31], [38]. Although the response rate of doxorubicin treating in FMC is favorable, doxorubicin can be nephrotoxic to cats and often cause gastrointestinal disturbance [9], [32].

Vinorelbine is a semisynthetic derivative of vinca alkaloids and widely distribution in most tissues, especially in lung. In human, vinorelbine can be used to treat breast cancer [5], [14], [23]. Based on previous studies in human and veterinary medicine, adverse events of vinorelbine are generally self-limiting and tolerable. Neutropenia is the most commonly documented adverse event and no obvious renal toxicity is noted in human and small animals [5], [8], [34].

For those cats with malignant and aggressive mammary carcinoma, pulmonary metastasis are the most fatal events. Vinorelbine may be appropriate to treat feline mammary carcinoma because there is high tissue and lung distribution of vinorelbine and has demonstrated activity against breast cancer in human. Therefore, the purpose of this study was to compare the efficacy and toxicity of vinorelbine and doxorubicin in FMC. In addition, possible prognostic factors were also investigated.

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Chapter 3 Materials and methods

3.1 Patient selection

Cats diagnosed with FMC histologically or cytologically and had been received doxorubicin or vinorelbine initially were enrolled to this study and divided into VRL group or DOX group. Criteria of cytological diagnosis of FMC were tumor cells presented with characteristics of epithelial cells and malignancy such as variable nuclear size, nuclear giant forms, high N:C ratio, variable numbers of nucleoli, abnormal nucleolar shape, or the presence of macronucleoli.

Cats in VRL group were all enrolled prospectively that were treated with vinorelbine as its first-line chemotherapy at National Taiwan University Veterinary Hospital (NTUVH) animal cancer treatment center since June, 2016. Signalment of age, breed, gender, neuter status and body weight; physical examination findings such as size, location and ulceration of tumor; results of clinical stage like thoracic radiography or computed tomography, lymph node status; information of histopathology such as tumor type, subtype, grade, surgical margin, and lymphatic and vascular invasion were all documented; variables of treatment included doses, dosage, interval of doses and whether patient had received rescue chemotherapy; response and adverse events of chemotherapy were recorded and analyzed.

Cats in DOX group were collected retrospectively. The medical records for cats diagnosed with FMC and treated with doxorubicin as its' first-line chemotherapy from 2014 to 2017 at NTUVH animal cancer treatment center were reviewed and assigned to DOX group. Cats were included in this study if complete medical records were available. Medical information and response of treatment were retrieved as mentioned above.

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3.2 Clinical stage

Cases were staged based on modified World Health Organization (WHO) clinical staging system for feline mammary carcinoma [38] (Table 2).

3.3 Tumor grade

According to veterinary histopathological grading system [28], tumors were classified as grade I (well-differentiated), grade II (moderately differentiated) or grade III (poor-differentiated) by pathologists in School of Veterinary Medicine, National Taiwan University.

3.4 Chemotherapy

3.4.1 VRL group

In VRL group, cats were prospectively treated with a starting dosage of 11.5 mg/m^2 vinorelbine (Vinorelbine Injection Concentrate, Hospira, Australia) once weekly for 4 weeks and followed by every other week administration for 4 times. After receiving 8 doses, cats were eligible to continue VRL treatment every 2 weeks at the owner's expense. Vinorelbine would be withdrawn when progression of disease was found, and rescue therapy was allowed. The prescribed dose was diluted in 0.9% NaCl to a concentration of 1 mg/ml and administered intravenously over 5 to 10 minutes. Complete blood count (CBC) and bio-chemistry profile were evaluated before each treatment. If neutrophil count <2000 cells/μL or other grade 3-5 toxicities were found, therapy was delayed until recovery, and the dosage of vinorelbine subsequently was reduced by 10% to 20%.

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3.4.2 DOX group

Doxorubicin (Adriblastina Rapid Dissolution, Pfizer, Italy) was administered intravenously infusion with a dosage range of 20 mg/m^2 to 25 mg/m^2, every two to four weeks. The regiments depended on clinicians' preference.

3.5 Response

Response to treatment was evaluated based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria [4]. Measurable tumors were measured with calipers before each treatment. Three-dimensional thoracic radiography was performed every 3-4 weeks in VRL group. In the case of multiple tumors, the longest two tumors of each organ were determined as the target lesions, and added the longest axis of target lesions to obtain the sum of diameters. Response was calculated by the formula: tumor response = [(post-treatment measurement – pre-treatment measurement) / pre-treatment measurement] x 100%, and then categorized as complete remission (CR; disappearance of all target lesions), partial remission (PR; >30% but <100% decrease in the sum of diameters of target lesions), progressive disease (PD; at least a 20% increase in the sum of diameters of target lesions or appearance of new lesion), stable disease (SD; neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD).

In the case with non-measurable disease initially but local recurrence or distant metastasis was documented during treatment, the case was classified as PD. If the case maintained with non-measurable disease at the beginning and the end of treatment, the case was defined as non-CR/non-PD.

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3.6 Toxicity

Toxicities were assessed based on results of hematology, bio-chemistry and patient history at each visit and categorized as grade 1-5 according to Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) [39] (Table 3).

3.7 Statistical analysis

All cases that met the inclusion criteria were included in the statistical analysis. Objective Response rate (ORR) was calculated only for patients with measurable disease and defined as the number of cases had been experienced CR or PR divided by the number of cases treated.

Biological response rate (BRR) was estimated for all patients and defined as the total number of cases had been experienced CR, PR, SD and Non-CR/non-PD divided by the number of cases treated.

Time to progression (TTP) was defined as the duration (in days) between the date of chemotherapy administered and development of disease progression such as local recurrence or distant metastasis. Overall survival time (OST) was defined as the time (in days) from the diagnosis of FMC was made to disease-related death. Duration of follow-up was defined as the period (in days) between the date of the first and the last visit to NTUVH.

To analyzed whether there was significant difference in tumor features and demographic distribution between VRL group and DOX group, Pearson's chi-square test was used in categorical data including gender, neuter status, with or without gross tumor, histological grade and subtype, location and ulceration of tumor, TNM stage, initially

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presenting with or without pleural effusion, had received rescue chemotherapy or not. However, when the expected value of a given cell in the comparison was less than five, Pearson's chi-square test was substitute by Fisher's exact test. Continuous data such as body weight, age and duration of follow-up in two group was compared by Mann-Whitney U test.

To compare ORR in two groups, Fisher's exact test was used. Pearson's chi-square test was used to compare BRR in two groups. Median TTP and OST was calculated by Kaplan–Meier method. Cases were censored for TTP analysis if lost to follow-up or if they were progression-free at the time of study closure. Cases were censored for survival analysis if still alive, lost to follow-up, or dead from unrelated disease. Differences of median TTP and median OST between two groups was assessed with the log-rank test.

Incidence of adverse event between two groups were analyzed by Pearson's chi-square test. The value of blood urea nitrogen (BUN) and serum creatinine before the first chemotherapy was defined as pre-chemotherapy BUN and pre-chemotherapy creatinine. The definition of post-chemotherapy BUN and creatinine was the value of BUN and creatinine after the last vinorelbine for VRL group and the last doxorubicin for DOX group. Pre-chemotherapy and post-chemotherapy BUN in each group were compared with paired t test. Likewise, pre-chemotherapy and post-chemotherapy creatinine in each group were compared with paired t test.

For all patients, univariate analysis to assess potential prognostic factors in TTP and OST was performed by the Kaplan-Meier method with log-rank test. Factors included body weight, age, neuter status; patient presented with macroscopic or microscopic disease when administered chemotherapy, ulceration and location of tumor; information of histopathology such as subtype, grade, with or without lymphatic and vascular invasion; previous surgical procedure; clinical stage and presented with or without pleural effusion;

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response of treatment; patient received rescue chemotherapy or not. Multivariate analysis included all univariate factors with $P \leq 0.2$ using the Cox proportional hazards model, and then the risk of TTP as well as OST were estimated with corresponding 95% confidence and *P* values. We set the cut off *P*-value of 0.2 instead of 0.05 to avoid increasing possibility of false positive and to avoid losing potentially significant factors [21].

All analyses were performed with SPSS v. 20 software. Statistical significance for all testing procedures was established at $P < 0.05$.

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Chapter 4 Results

4.1 Demography

4.1.1 Characteristics

Twenty-six cats met the inclusion criteria. Seven cats were assigned to VRL group and 19 cats assigned to DOX group. There were 17 (65%) mixed breeds, 5 (19%) Persians, 2 (8%) American short hairs, 1 (4%) British short hair and 1 (4%) Abyssinian. Median age was 12 years (range 6-14 years) in VRL group and 12 years (range 7-15 years) in DOX group. Median body weight was 4.06 kg (range 2.66-4.86 kg) in VRL group and 3.78 kg (range 2.56-5.82 kg) in DOX group. All patients were female and naïve to chemotherapy. In VRL group, there were 6 (85.7%) neutered females and 1 (14.3%) intact female when diagnosed with FMC. Eleven (57.9%) neutered females and 8 (42.1%) intact females were in DOX group. No significant differences were observed in age, gender (neuter status), body weight or breed. (Table 4)

4.1.2 Tumor features

Six cats were diagnosed with FMC based on cytological findings, while 20 cats were diagnosed histologically including 13 (65%) adenocarcinomas and 7 (35%) carcinomas. Four (57.1%) cats in VRL group and 11 (57.9%) cats in DOX group had gross tumors when they received chemotherapy. Tumors located unilaterally for 5 (71.4%) cats in VRL group and 11 (57.9%) cats in DOX group. Ulceration of tumors were found in 17 cats (3 were in VRL group and 14 were in DOX group). Twenty-five cats had received surgery, but only 4 cases in VRL group and 9 cases in DOX had information about surgical margin and lymphatic and vascular invasion. Dirty margin and lymphatic and vascular invasion of tumor cells were noted in 2 (50%) cases of VRL group; three (33.3%) cases had dirty

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surgical margin and 8 (88.9%) cases presented with lymphatic and vascular invasion in DOX group. The histological grade of FMC was determined in 12 cases. The distribution of tumor grade for VRL group was 2 (40%) grade 3 tumors, 2 (40%) grade 2 tumors and 1 (20%) grade 1 tumor. There were 3 (42.9%) grade 3 tumors, 2 (28.6%) grade 2 tumors and 2 (28.6%) grade 1 tumors for DOX group. Four (57.1%) cats in VRL group and 11 (57.9%) cats in DOX group had gross tumors when they received chemotherapy. No significant differences were noted in percentage of patients with gross disease, tumor type, ulceration of tumor, percentage of dirty margin and lymphatic and vascular invasion, distribution of tumor grade between two groups. (Table 5)

4.1.3 Clinical stage

In VRL group, 5 (71.4%) cats had tumors >3 cm in diameter at the time of initial diagnosis; 1 (14.3%) cat had tumor in 2-3cm in diameter and 1 (14.3%) had tumor <2 cm in diameter. In DOX group, 11 (61.1%) cats had tumors >3 cm; 2 (11.1%) cats had tumors in 2-3cm in diameter and 5 (27.8%) had tumor <2 cm in diameter at the time of initial diagnosis. Seventeen cats had been evaluated lymph node status, and 2/4 (33.3%) cats in VRL group and 10/11 (90.9%) cats in DOX group had lymph node metastasis. Pulmonary metastasis was found in 4 (57.1%) cats in VRL group and 10 (52.6%) cats in DOX group. One (14.3%) cat of VRL group and two (10.5%) cats of DOX group had pleural effusion. Twenty-four cats had been fully staged. The distribution of clinical stage for VRL group was 4 (57.1%) stage 4, 1 (14.3%) stage 3 and 2 (28.6%) stage 1. In DOX group, the distribution of clinical stage was 10 (58.8%) stage 4, 6 (35.3%) stage 3, and 1 (5.9%) stage 1. No case was classified as stage 2 in both groups. Significant difference was observed in only in lymph node status (P =0.028). (Table 6)

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4.2 Treatment of VRL group

Forty-four doses of vinorelbine were administered. A median of 8 doses (range 2-9 doses) were given in 7 cats. Dose escalation was performed once in one cat. All cats experienced dose reduction. Median dosage was 10.35 mg/m^2 (range 7.36-12.6 mg/m^2) (Table 7). Three cats (57.1%) had received rescue chemotherapy when progression was found. One cat was administered 3 doses of doxorubicin and 1 dose of cyclophosphamide; another cat received 1 dose of doxorubicin but stopped rescue chemotherapy due to severe adverse event of anorexia; the other cat was also given 1 dose of doxorubicin and stopped rescue chemotherapy because of progression disease.

4.3 Treatment of DOX group

Forty doses of doxorubicin were given. A median of 2 doses (range 1-4 doses) was administered in 19 cats. Three cats experienced dose escalation and four cats experienced dose reduction. Median dosage was 25 mg/m^2 (range 20-25 mg/m^2) and median cumulative dosage was 45 mg/m^2 (range 20-100 mg/m^2) (Table 8). Three cats (15.8%) had received rescue chemotherapy. One cat was given one dose of cyclophosphamide; another cat received 2 doses of cyclophosphamide; the other cat was administered vinorelbine for 1 dose.

Total number of cats received rescue chemotherapy was not statistic difference between VRL group and DOX group (P =0.146).

4.4 Outcome

4.4.1 Response

In VRL group, 4 cases had measurable tumors. Of these 4 cats, 1 experienced CR, 2

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experienced PR and 1 achieved SD. Objective response rate of VRL group was 75%. Of the 3 cats without measurable tumors, all of them maintained Non-CR/non-PD when treatment finished. Biological response rate for all 7 cats in VRL group was 100% (Table 9).

Eleven cases had measurable tumors in DOX group. None achieved CR. Two cases experienced PR, 2 cases achieved SD and 7 cases were PD. Response rate of DOX group was 18.2%. For 8 cats without measurable disease, PD was found in 4 cases including 2 local recurrences and 2 pulmonary metastases; 4 cases maintained Non-CR/non-PD. Biological response rate for cats in DOX group was 42%.

No statistic difference was observed in objective response distribution for patients with measurable disease between 2 groups (P =0.058) or patients with non-measurable disease between 2 groups (P =0.077). However, a significant difference (P =0.008) was noted when comparing biological response rate for all patients between two groups (Table 9).

4.4.2 Time to progression and overall survival time

Median duration of follow-up was 160 days (range 37-457 days) for VRL group and 196 days (range 21-593 days) for DOX group (P =0.692).

One case in VRL group was progression free at Day 35 when the study completed and censored to estimate TTP. Two cases in DOX group were lost to follow-up at Day 62 and Day 49, so these two cases were also censored to estimate TTP. The Kaplan-Meier median TTP for VRL group and DOX was 115 days and 102 days respectively. No significance was found in median TTP between two groups (P =0.949) (Figure 1).

At the completion of the study, 12 cats were censored from the Kaplan-Meier survival analysis (3 cats in VRL group are still alive at Day 70, 160 and 354 when the

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study completed; 9 cats in DOX group were lost to follow-up at Day 38, 51, 62, 67, 196, 274, 280, 301 and 570). The Kaplan-Meier OST was 352 days for VRL group and 284 days for DOX group and no significant difference was observed (P =0.948) (Figure 2). Time to progression and overall survival time for two groups were summarized in Table 10.

4.5 Toxicity

Neutropenia was the most common adverse event in VRL group. All 7 cats in VRL group experienced neutropenia. Nineteen (43.2%) episodes of neutropenia were observed in 44 doses of VRL and all were non-febrile neutropenia. No evidence of sepsis or clinical signs related to neutropenia was found. There were 6 (13.6%) episodes of grade 1, 5 (11.4%) episodes of grade 2, 6 (13.6%) episodes of grade 3 and 2 (4.5%) episodes of grade 4 neutropenia. Both grade 4 neutropenia episodes happened to the same cat at the dosage of 12.6 mg/m^2 and 11.4 mg/m^2. On the other hand, there was no neutropenia episode in 40 doses of doxorubicin for DOX group. Hence, significant difference of neutropenia was noted (P <0.001) (Table 11).

Gastrointestinal (GI) toxicity was the most common adverse event in DOX group. Cats in DOX group had 19 (47.5%) episodes of vomiting after 40 doses of doxorubicin, and there were 16 episodes of grade 1 and 3 episodes of grade 2 vomiting. As in VRL group, there were only 8 (18.2%) episodes of vomiting after 44 doses of vinorelbine, and all were grade 1. Significance was found in the total number of episodes of vomiting between two groups (P =0.004). Nineteen (47.5%) anorexia incidents were documented in DOX group and there were 5 grade 1 incidents, 13 grade 2 incidents and 1 grade 3 incidents. Only 5 (11.4%) incidents of anorexia were observed in VRL group and there

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were 3 grade 1 incidents and 2 grade 2 incidents. Total number of anorexia episodes and the incidence of grade 2 anorexia were both significant higher in DOX group (P <0.001 and P =0.001, respectively). Three episodes of diarrhea occurred in VRL group and 4 episodes of diarrhea occurred in DOX group. All episodes of diarrhea were grade 1 and no significance was observed between two groups (P =0.598) (Table 11).

A mean of pre-treatment BUN was 23.8 ± 3.93 mg/dL (median 25 mg/dL, range 16-28 mg/dL) in VRL group and 22.6 ± 6.22 mg/dL (median 21 mg/dL, range 13-36 mg/dL) in DOX group. A mean of post-treatment BUN was 27.4 ± 7.74 mg/dL (median 25 mg/dL, range 20-42 mg/dL) in VRL group and 31.7 ± 21.7 mg/dL (median 24 mg/dL, range 14-107 mg/dL) in DOX group. When using paired sample t test to compare the change of BUN between pre- and post-treatment in two groups, there was no difference in VRL group (P =0.226) but nearly achieved significance in DOX group (P =0.059). A mean of pre-treatment creatinine was 1.52 ± 0.40 mg/dL (median 1.50 mg/dL, range 1.10-2.30 mg/dL) for VRL group and 1.38 ± 0.25 mg/dL (median 1.40 mg/dL, range 1.00-2.00 mg/dL) for DOX group. A mean of post-treatment creatinine was 1.71 ± 0.74 mg/dL (median 1.50 mg/dL, range 1.10-3.30 mg/dL) in VRL group and 1.60 ± 0.56 mg/dL (median 1.4 mg/dL, range 1.10-3.20 mg/dL) in DOX group. Paired sample t test was also used to compare the change of creatinine from pre-treatment to post treatment in two groups. There was significant elevation of creatinine in DOX group after treatments (P =0.033) but not in VRL group (P =0.258).

All cats in VRL group experienced dose reductions. Median number of dose reduction in VRL group was 2 times (range 1-3 times) and all dose reductions were due to neutropenia. In contrast, dose reduction was performed in four cats for DOX group. Those four cats all experienced 1 time of dose reduction. The reasons of dose reduction were GI toxicities in 1 cat and uncertain in the other 3 cats.

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4.6 Prognostic factors analysis for all patients

4.6.1 Prognostic factors analysis for TTP

Univariate analysis of possible prognostic factors for TTP for all patients was performed (Table 12 and Table 13). Factors included body weight (< or ≥ median body weight), age (< or ≥ median age), neuter status (neutered or intact), gross tumor (patient presented with macroscopic or microscopic disease when administered chemotherapy), ulceration, tumor location (unilateral or bilateral), histological subtype (cribriform, tubulopapillary or combination type), tumor grade, with or without lymphatic and vascular invasion, previous surgical procedure (regional or unilateral excision), clinical stage and presented with or without pleural effusion, biological response of treatment (responders or non-responders), patient received rescue chemotherapy or not.

Significant differences were observed only in factors of neuter status and biological response. Neutered female cats had significant shorter median TTP than intact female cats (63 days versus 119 days, P =0.021) (Figure 3). Biological non-responders also had significant shorter median TTP (28 days versus 119 days, P =0.008) (Figure 4). Patients presented with gross tumor when received chemotherapy, ulcerative tumors or distant metastasis had relatively shorter median TTP but not significant (P =0.141, P =0.080 and P =0.15, respectively). Although these 3 factors were not statistic difference, the P-values of these three factors were less than 0.2. Hence, neuter status, biological response, gross tumor, ulceration and M stage were evaluated in multivariate analysis.

Results of multivariate analysis with hazards ratio (HR) and 95% confidence interval (95% CI) of factors for TTP were summarized in Table 14. Neuter status and biological response remained statistic significant (P =0.014 and P =0.000, respectively). Hazards ratio for neutered cats and non-responders were 5.377 (95% CI, 1.406-20.562) and 19.397

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(95% CI, 3.680-102.249) respectively. Factor of M stage was nearly significant (HR, 8.730; 95% CI, 0.868-87.811; *P* =0.066).

4.6.2 Prognostic factors analysis for OST

Factors previously mentioned in univariate analysis for TTP were also analyzed for OST. In addition, the factor of patient received rescue chemotherapy or not was assess for possible prognostic factor as well.

In univariate analysis of possible prognostic factors for OST, only biological response achieved significance (*P* =0.012) (Figure 7). Non-biological responders had significant shorter median OST than responders (232 days versus 446 days). Location of tumors was nearly significant. Tumors located bilaterally had relatively shorter OST (254 days versus 446 days, *P* =0.069) (Figure 6). Besides, patients presented with body weight ≥3.87 kg, age <12 years, ulcerative tumors, lymph node metastasis and administered with rescue chemotherapy were also had relative shorter median OST, but not significant (*P* =0.151, *P* =0.152, *P* =0.196, *P* =0.166 and *P* =0.159, respectively) (Table 15 and Table 16). Factors with *P*-value less than 0.2 in univariate analysis included biological response, tumor location, body weight, age, ulceration, lymph node status and received rescue therapy or not. All these factors with *P*-value less than 0.2 were evaluated in multivariate analysis except lymph node status and summarized in Table 17. Because there were too many missing data of lymph node status (n =9, 35%), this factor was censored in multivariate analysis to avoid interfering results.

Ulceration of tumors and biological response were the only two factors that achieved statistical significance in multivariate analysis for OST. Hazards ratio was 5.192 (95% CI, 1.137-23.706; *P* =0.034) for patients with ulceration of tumor and 4.027 (95% CI, 1.004-16.152; *P* =0.049) for non-responders.

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Chapter 5 Discussion

5.1 Efficacy and toxicity

For the treatment of FMC, adjuvant chemotherapy is usually recommended in cases with tumor showing evidence of lymphatic or blood vessels invasion, while some recommend chemotherapy for all cases [6]. Doxorubicin is the mostly used chemotherapy agent in canine and feline mammary carcinoma. To the author's knowledge, this is the first study to compare the efficacy and the toxicities between vinorelbine and doxorubicin in FMC. In the present study, 7 cats received vinorelbine for first-line adjuvant chemotherapy and 19 cats were administered with doxorubicin as their first chemotherapy agent. Although the response rate of VRL group was higher than DOX group (75% versus 18%), there is no significant difference in median TTP for two groups (115 days for VRL group and 102 days for DOX group, P =0.949). However, the nature of this study design may cause biased results. This study was not a randomized controlled trial. Cats in VRL group were treated with vinorelbine prospectively, while cats in DOX group were enrolled to this study retrospectively. In addition, most cats in DOX group were administered doxorubicin every 3 to 4 weeks, but cats in VRL group were treated with vinorelbine every 1 to 2 weeks. Hence, cats in VRL group had much more frequent follow-up, and therefore any progression events such as local recurrence, distant metastasis or even pleural effusion would be detected earlier. On the other hand, because of the nature of retrospective design of DOX group, there was lack of standard and regular follow-up schedule, which would result in overestimating TTP for DOX group. This might be the reason why there was much higher response rate in VRL group but similar TTP in two groups. Because TTP is not affected by rescue or subsequent therapy, it is an important endpoint to compare the efficacy between different therapies. As the result, the efficacy

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of vinorelbine treated in FMC was similar to doxorubicin. In the aspect of survival time, median OST was numerically longer for VRL group (352 days) than DOX group (284 days), although there was no statistical significance (P =0.948). This could be due to the small sample size and relative high percentage of patients in VRL received rescue chemotherapy.

According to a research by Novosad et al [31], 67 cats with FMC underwent adjunctive doxorubicin treatment and the disease-free interval was 183 days and the overall survival time was 331 days for cats with pulmonary metastasis. Mauldin et al used doxorubicin-based chemotherapy to treat 14 cats with advanced stage of mammary adenocarcinoma; median survival time was 90 days in that study [25]. These results indicated that the outcome of our study was comparable to previous studies. In a study by McNeill et al [26], disease-free interval (DFI) was 676 days and OST was 848 days for 36 cats with mammary carcinoma received adjuvant doxorubicin therapy after surgery; both DFI and OST in that study were much longer than VRL group or DOX group in our study or even longer than previous studies. Nevertheless, in that study, only 23% cats had evidence of lymph node metastasis and all of which were removed at the time of surgery. In contrast, there was almost 60% cats had pulmonary metastasis in both groups in our study and this would result in a relative unfavorable outcome.

In the present study, gastrointestinal toxicities were the most common adverse events in DOX group and the incidence of vomiting (48%) and anorexia (48%) were both significant higher than VRL group. Besides, GI toxicity was also the main reason of dose reduction for DOX group. Most of cats (70%) in DOX group received 25 mg/m^2 as their starting dosage and three cats experienced dose reduction. Some oncologists may use 1 mg/kg (approximately 20 mg/m^2) as starting dosage in cats to reduce the incidence of toxicity. However, a decreased of dosage may also decrease the efficacy of chemotherapy.

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In a previous study [36], different dosing schemes of doxorubicin were used to treat tumor-bearing cats and compare the toxicities. Vomiting and anorexia were the most common reported complaints in that study. There were 24 episodes of vomiting in 129 doses (18.6%) for cats received 20 mg/m^2 doxorubicin, and 12 episodes of vomiting in 58 doses (20.6%) for cats administered 25 mg/m^2 doxorubicin. Most (50% to 67%) of vomiting episodes were classified as grade 2 in both groups. The incidence of anorexia was 10.9% and 10.3% for 20 mg/m^2 and 25 mg/m^2 doxorubicin, respectively. Most of anorexic episodes (about 60%) were classified as grade 1 in both groups. The results of that study suggested that a lower dosage of doxorubicin may not be associated with reducing risk of GI toxicity. Despite the fact that there was similar dosage in our study and the previous study, the incidence of vomiting and anorexia were both higher. The reason for this difference might be that most of patients in our study were advanced stage. Patients with advanced stage may relatively have poor nutrition or hydration status and caused higher incidence of chemotherapy-related toxicities.

Neutropenia was the most common adverse event for VRL group in contrast. The incidence of neutropenia was 44% for VRL group and significant higher than DOX group (0%). All cats received vinorelbine with a starting dosage of 11.5 mg/m^2, and all cats experienced neutropenia during their therapies. Neutropenia occurred after the first dose of vinorelbine in 4 cats and after the second dose in 2 cats. Dose escalation (12.6 mg/m^2) of second dose of vinorelbine was performed in only one cat, but grade 4 neutropenia then happened to this cat. The dosage of third dose was therefore reduced to 11.4 mg/m^2 for this cat; however, neutropenia still occurred at this dosage. Based on previous phase I study [34], the maximum tolerated dose for tumor-bearing cats was 11.5 mg/m^2 and recommended as starting dosage. Neutropenia was also a common type of adverse events in that study, and 2 cats experienced worsening grades of neutropenia at de-escalated

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dosages. One possible explanation mentioned in that study was that there was cumulative dosing and detrimental effects on the bone marrow. Another possible explanation was that there might be a double neutrophil nadir, which was similar to lomustine, an alkylating nitrosourea compound commonly used in treating lymphoma and histiocytic sarcoma in small animals. Although the incidence of neutropenia caused by vinorelbine was high in our study, none of these cats experienced sepsis and all of the neutrophil counts normalized within 7 days. As for DOX group, the incidence of neutropenia was 0. Incidence of neutropenia was 12% for cats treated with 25 mg/m^2 doxorubicin in a previous study by Reiman et al., and 71% of neutropenic episodes happened in 0-14 days after the treatment. The nadir (time of the lowest neutrophil count) of doxorubicin usually occurs at 5 to 10 days after the treatment in small animals [16], [19]. All DOX group cats in our study were administered doxorubicin every 3-4 weeks and CBC were evaluated at the day of each treatment. Therefore, clinicians might miss the nadir and resulted in an underestimated incidence of neutropenia.

Renal injury was an important and major concern when giving and dosing chemotherapy, especially using doxorubicin in cats. Doxorubicin can cause renal tubular necrosis, renal infarction or interstitial nephritis in cats and lead to decreasing creatinine clearance [32]. Nephrotoxicity can be seen with cumulative doxorubicin dosages of 130–320 mg/m^2 [32]. Although there was significant elevation of creatinine in DOX group after treatments in our study, the changes of creatinine level were not obvious. The possible explanation of rare azotemia episodes in DOX group was that the cumulative dosage in our study was much lower than previous studies (median cumulative dosage: 45 mg/m^2, range 20-100 mg/m^2).

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5.2 Prognostic factors

Although the tumor behavior of FMC is generally aggressive, time to progression and overall survival time can be quite various. Higher T stage, presented with vascular or lymphatic tumor invasion, status of metastasis and histopathological subtype of solid or cribriform are negative prognostic factors to time to progression in previous research [6], [31]. Numerous studies have identified factors that will influence survival times, including tumor size, WHO stage, histopathological grade, proliferation profile and surgical approach [6], [24], [27], [37].

In this study, neuter status and biological response to chemotherapy were the only two factors that significantly influence TTP in univariate analysis, and these two factors remained significance in multivariate analysis. It was not surprising that responders of chemotherapy had longer TTP than non-responders. But it was interesting that intact female cats in our study had longer TTP compared to neutered female cats (119 days versus 63 days; HR, 5.377; 95% CI, 1.406-20.562; P =0.021 and 0.014 for univariate and multivariate analysis, respectively). All but one intact female cats had undergone tumor removal with concomitant OHE. Sex hormones are associated with mammary tumor development in small animals and humans [17], [38], [40]. A study by Overley et al showed that cats spayed before 1 year of age have a significantly decreased risk of developing mammary carcinoma [33]. Estrogen receptor (ER) and progesterone receptor (P4R) are two main hormonal receptors implicated in mammary tumor development. Many previous studies have documented that most feline mammary carcinomas are ER and P4R negative, although slightly more than one-third are P4R positive. Dogs with ER-positive or P4R-positive mammary carcinoma were significantly associated with low proliferation index and histopathological grade 1 (low grade) [17]. One study indicated

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that expression of hormonal receptors of FMC is associated with lower malignancy and better prognosis [22], which is same as human medicine. In addition, a significant correlation of hormonal receptor (ER and P4R) positivity with absence of ovariectomy has been reported in both dogs and cats [17], [22]. Intact female dogs with ER-positive tumors or with increased peri-surgical serum 17β-estradiol (E2) represent a subset of dogs with mammary carcinomas likely to benefit from OHE [17], but these results are not established in feline medicine yet. Based on those studies, ER and P4R expression may be higher for intact female cats in our study and most of which underwent concomitant OHE and lead to longer TTP. Further screen test for ER and P4R expression and randomized controlled studies will be needed to confirm the hypothesis.

Tumor size (T stage), clinical stage, lymphatic or vascular invasion, histopathological subtype, grade of tumor and surgical approach were all not associated with TTP in our study. This result was opposed to previous studies. One possible explanation was that there were small and uneven distribution samples in the study. A non-randomized and non-prospective study usually has selection bias of cases. Most cases were advanced TNM stage and presented with lymphatic or vascular invasion in this study. Another possible reason was that not all cases had complete information of histopathology result; approximately half of cases lacked information for surgical margin, tumor grade and subtype.

In the univariate analysis of prognostic factors for survival time, only biological response to chemotherapy was significant and this factor remained significant in multivariate analysis. Biological responders had longer survival times than non-responders (446 days versus 232 days; HR, 4.027; 95% CI, 1.004-16.152; P =0.012 and 0.049 for univariate and multivariate analysis, respectively). Based on previous studies, the objective response rate was about 40% to 50% for cats with mammary carcinoma

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treated with doxorubicin-based chemotherapy, but this relatively high response rate did not reflect on survival benefits in those retrospective studies [38]. However, no further analysis was done to compare the survival time between responders versus non-responders and between cats with macroscopic disease versus microscopic disease in those studies. In our study, cats with biological response to chemotherapy got significant longer survival time. Since approximate half of biological responders (46%) were Non-CR/non-PD, which were cats with microscopic, non-measurable disease initially and maintained with non-measurable disease at the end of treatment, one possibility was that cats with microscopic disease initially may have longer survival time, and lead to longer survival time for whole biological response group. Nevertheless, no significant difference of survival time between cats with macroscopic disease and with microscopic disease was observed in our analysis. Thus, our results demonstrated that cats with biological response had survival benefits.

Cats with unilateral tumors was trending toward significance when compared for cats with bilateral tumors in univariate analysis (446 days versus 254 days, P =0.069). Complete and unilateral mastectomy in cats with mammary carcinoma showed a significantly better tumor control in a previous study [20]. As a result, bilateral mastectomy would be recommended for cats with bilateral mammary gland tumors. However, bilateral mastectomy performed as a single procedure can result in wound dehiscence because of high skin tension. Most surgeons therefore tend to undergo staged unilateral mastectomy, but metastasis may occur between twice surgeries. In addition, the cost and duration of recovery of bilateral mastectomy would be higher and longer, and those factors may decrease owners' willingness of aggressive surgery. It was also suspected that there might be more aggressive tumor behavior for bilateral mammary tumors than unilateral tumors. Rare studies in veterinary medicine compared the survival

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time between cats with bilateral and with unilateral mammary carcinoma. Contralateral mammary carcinoma can be either a metastatic lesion or the second primary cancer, and occurs either synchronously or metachronously. According to results of human medicine, several studies indicated women with bilateral breast cancer had worse prognosis than women with unilateral breast cancer [7]. In a large cohort study by Hartman et al [11], women with synchronous bilateral breast cancer had a higher mortality from breast cancer than women with unilateral disease. This evidence in human may be another reason why cats with bilateral mammary tumor were trending toward shorter survival time in our study.

It is interesting that ulceration of tumors was not significant to survival in univariate analysis (P =0.196) but this factor became significant in multivariate analysis (HR, 5.192; 95% CI, 1.137-23.706; P =0.034). Ulceration of tumors were associated to aggressive tumor behavior and high histopathological grade [27], which is an important prognostic factor of survival time. In addition, ulceration of tumors would cause pain, poor skin hygiene or infection and lead to poor quality of life as well as early euthanasia.

Tumor size, status of metastasis and even grading were not significant prognostic factors for survival time in our study, while those factors were strongly correlated to prognosis in other reports. As mentioned previously, lack of histopathological diagnosis in some cases, most of patients were advanced stage and high grade, small sample size and the nature defects of retrospective were the possible reasons.

5.3 Limitations

There were lots of limitations in this study. First of all, the sample size was too small; only 26 cats were enrolled to our study. Second, it was not a prospective and randomized

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trial to make our results more persuasive. Cats in DOX group were lack of a standard protocol of treatment and scheme of follow-up, which would interfere the results of efficacy. Due to not all cases had histopathological diagnosis, some prognostic factors cannot be evaluated for all patients.

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Chapter 6 Conclusion

Cats received vinorelbine presented longer time to progression and overall survival time, although there were not statistical significant. Incidence of gastrointestinal toxicity was significant lower in cats treated with vinorelbine when compared to those receiving doxorubicin, and neutropenia was the most common documented adverse event in cats administered vinorelbine. In conclusion, cats with FMC treated with vinorelbine had similar efficacy but less GI toxicity compared to cats treated with doxorubicin; therefore, vinorelbine may be an appropriate alternative agent to be used in treating FMC. Neuter status, ulceration of tumor and biological response were prognostic factors in the study.

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Figures

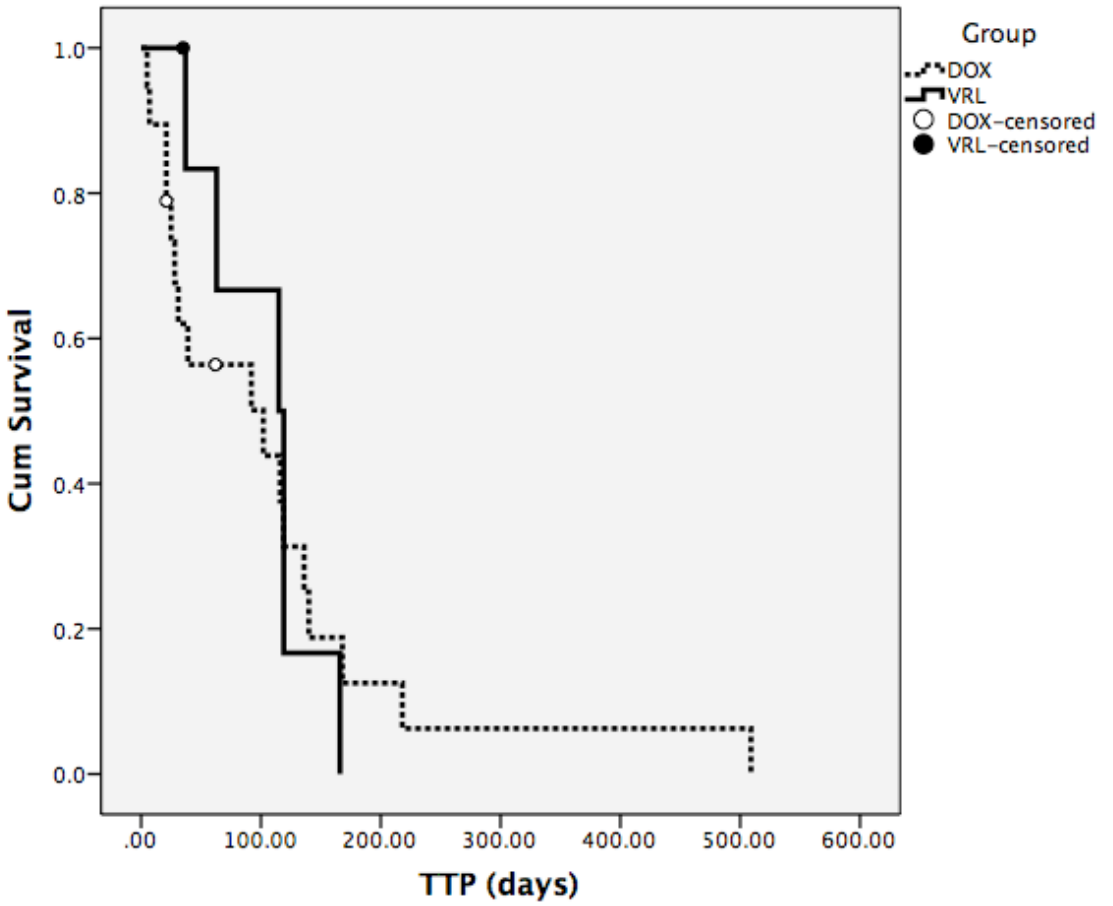

Figure 1. The Kaplan-Meier curve for time to progression (TTP) of the two groups.

The median TTP for VRL group (solid line, n =7) and DOX (dashed line, n =19) was 115 days and 102 days respectively. No significance was found in median TTP between two groups (*P* =0.949)

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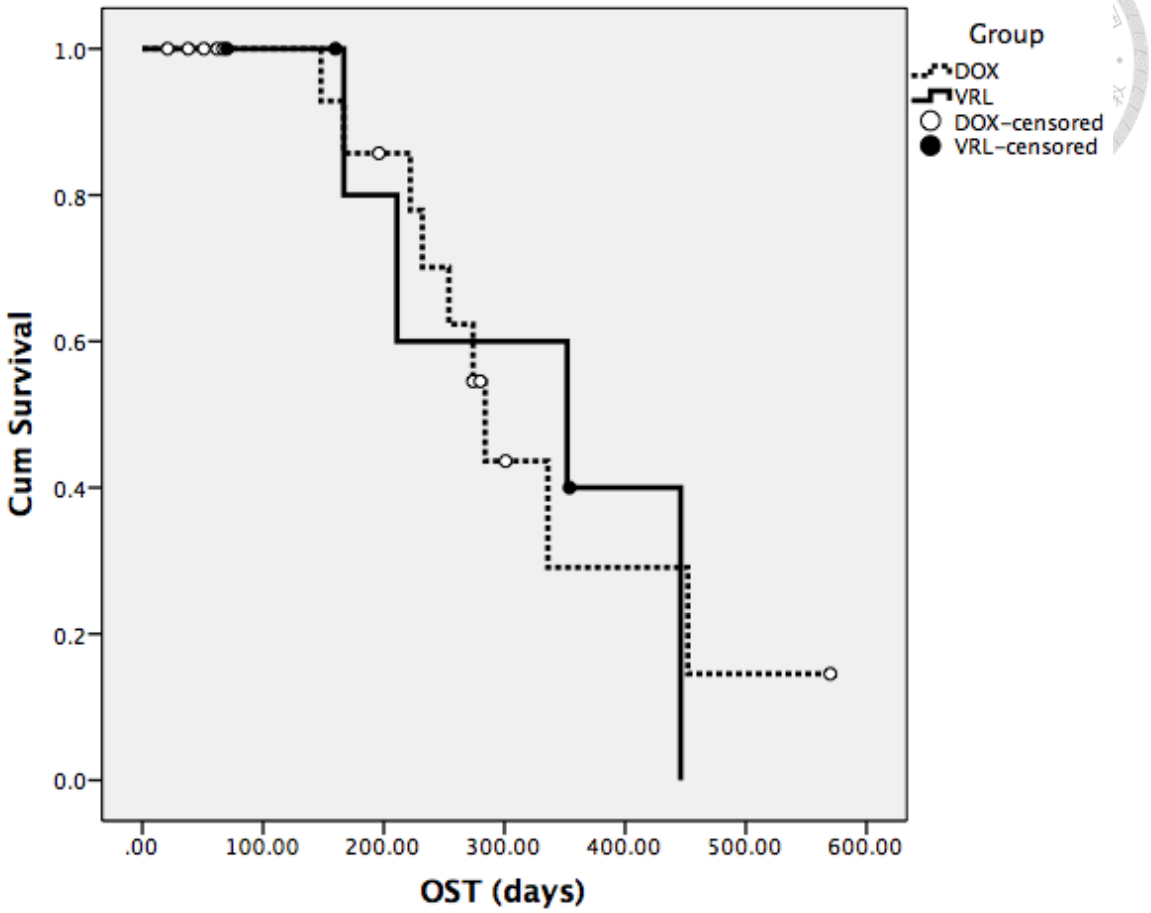

Figure 2. The Kaplan-Meier curve for overall survival time (OST) of the two groups.

The median OST was 352 days for VRL group (solid line, n =7) and 284 days for DOX group (dashed line, n =19). No significant difference was observed (*P* =0.948)

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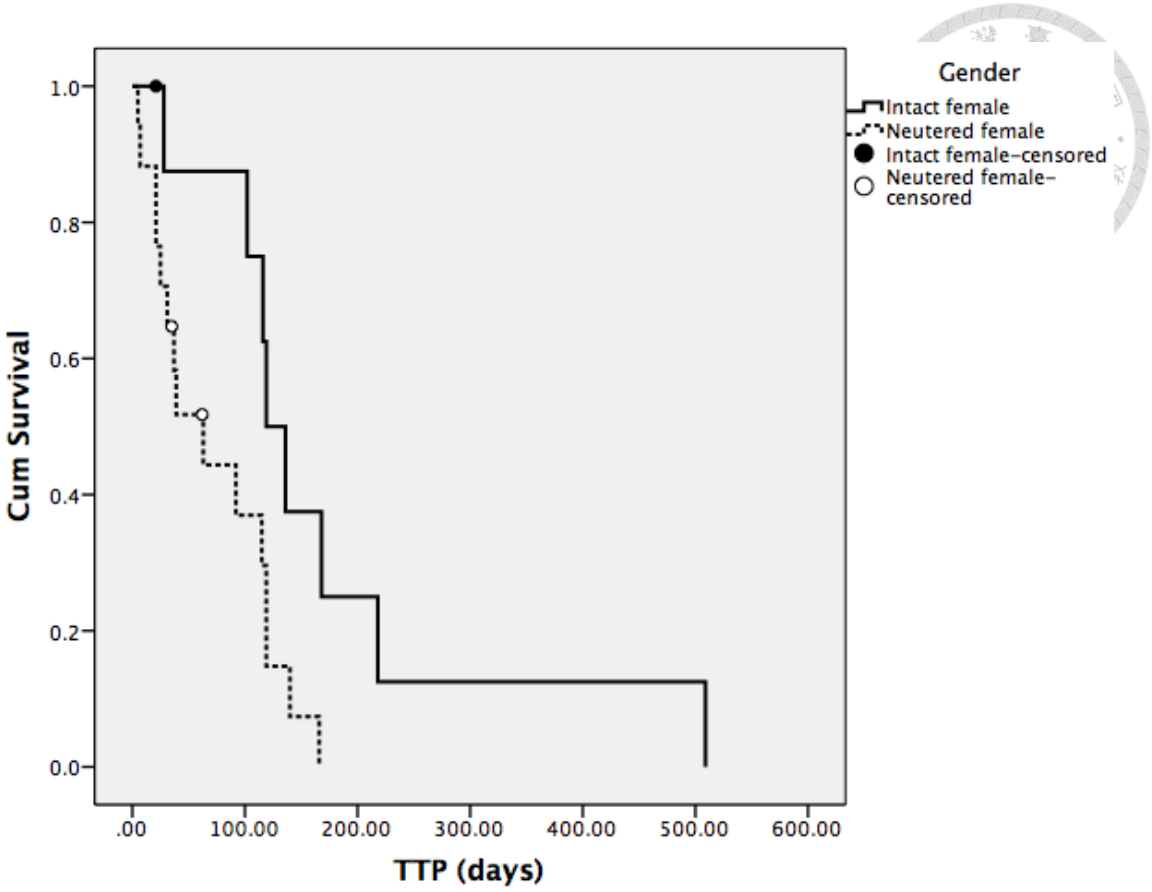

Figure 3. The Kaplan-Meier curve for time to progression (TTP) between gender.

The median TTP was 119 days for intact female cats (solid line, n =9) and 63 days for neutered cats (dashed line, n =17). (P =0.021)

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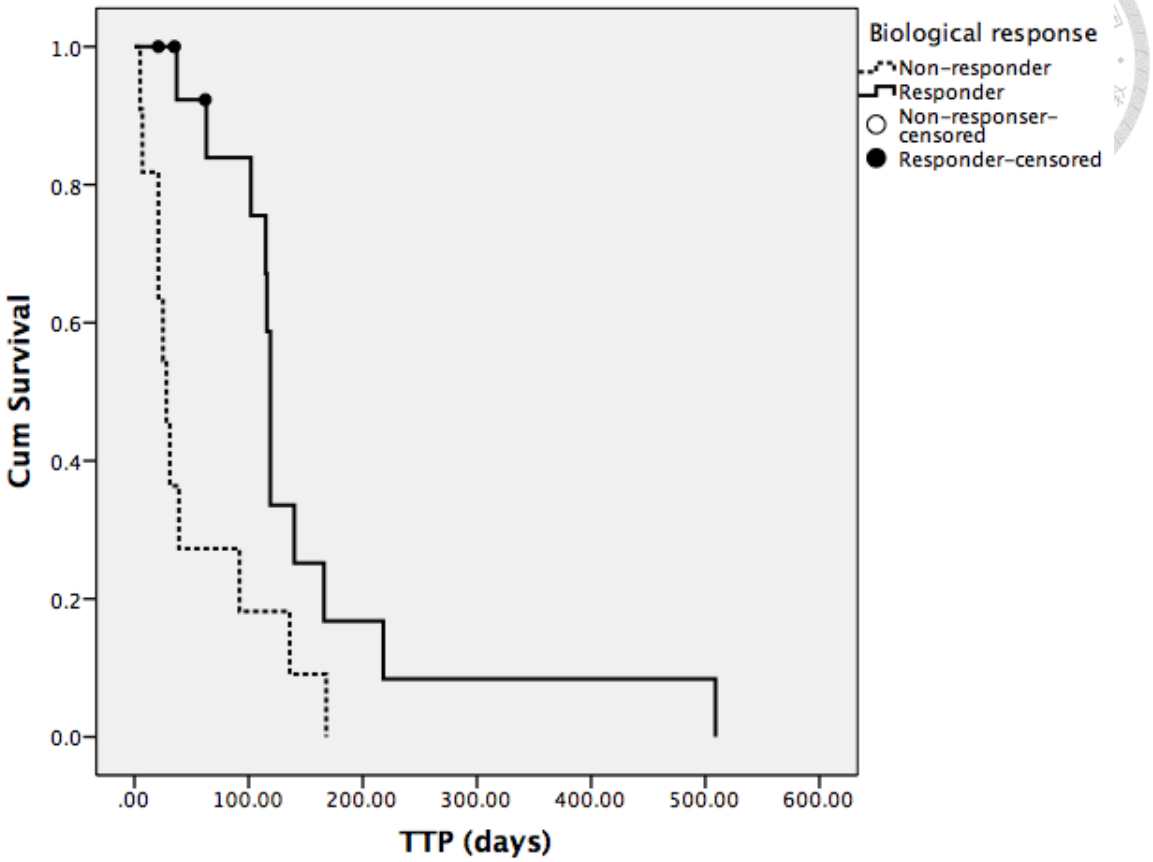

Figure 4. The Kaplan-Meier curve for time to progression (TTP) among different biological responses.

The median TTP was 119 days for responders (solid line, n =15) and 28 days for non-responders (dashed line, n =11). (*P* =0.008)

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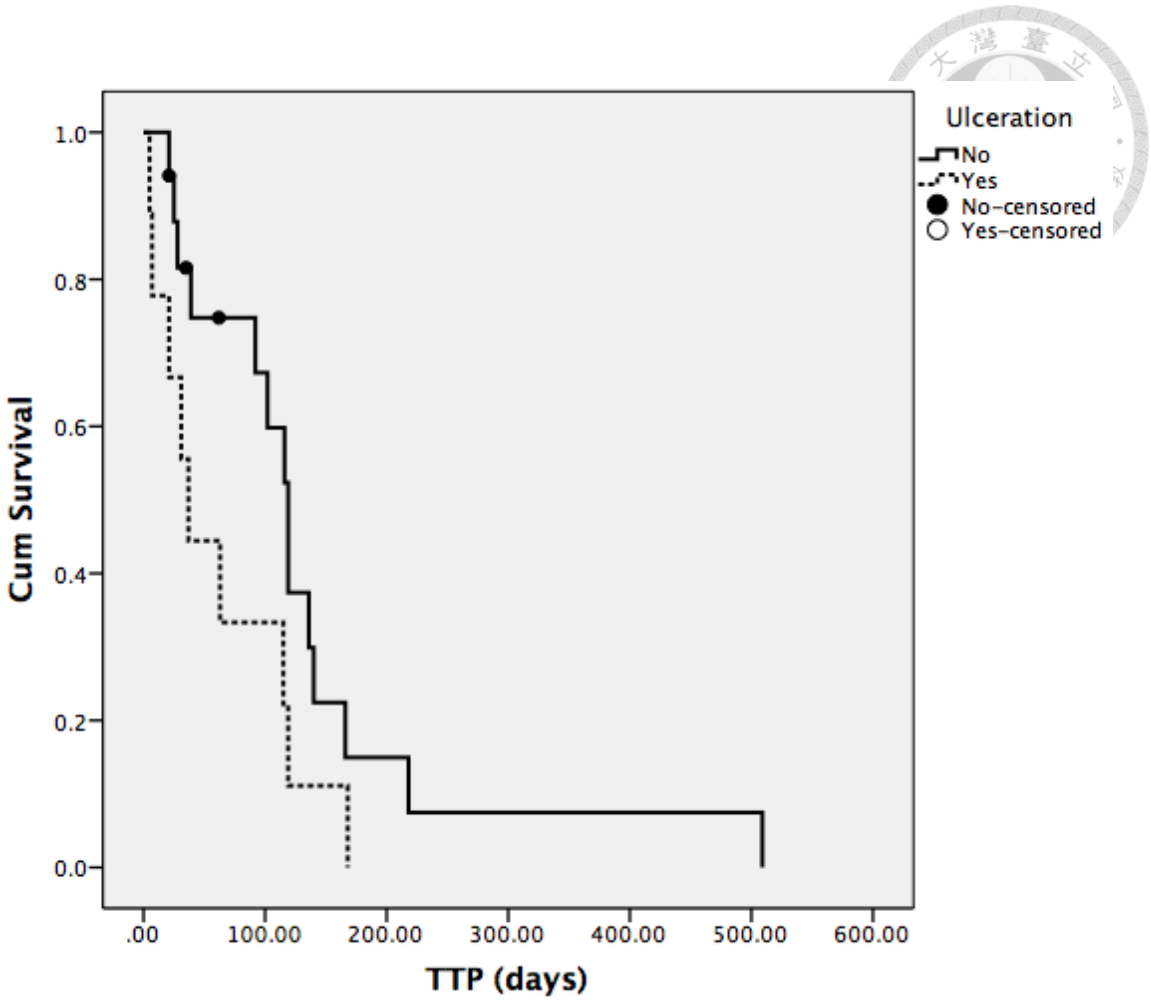

Figure 5. The Kaplan-Meier curve of time to progression (TTP) for ulceration group.

The median TTP was 119 days for cats without ulcerative tumors (solid line, n =17) and 37 days for cats with ulcerative tumors (dashed line, n =9). (P =0.080)

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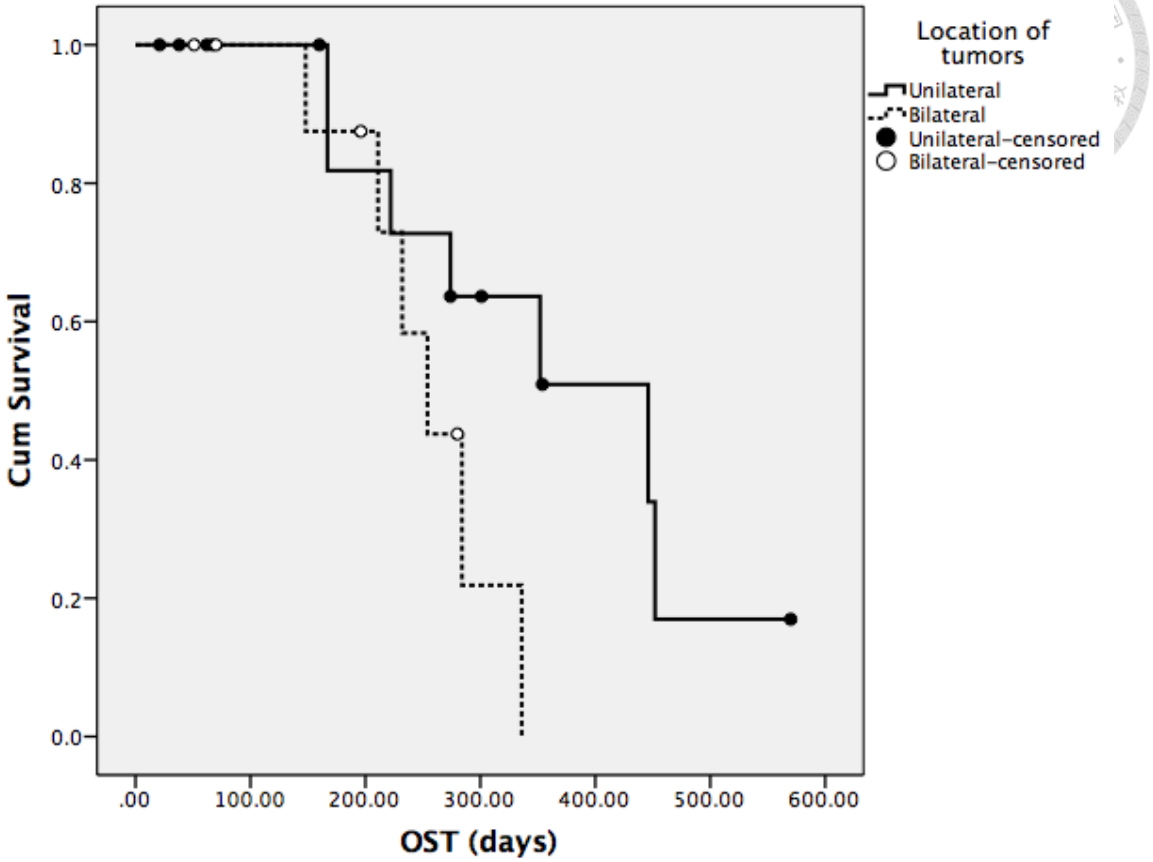

Figure 6. The Kaplan-Meier curve of overall survival time (OST) for different tumor distribution groups.

The median OST was 446 days for cats with unilateral tumors (solid line, n =16) and 254 days for cats with bilateral tumors (dashed line, n =10). (*P* =0.069)

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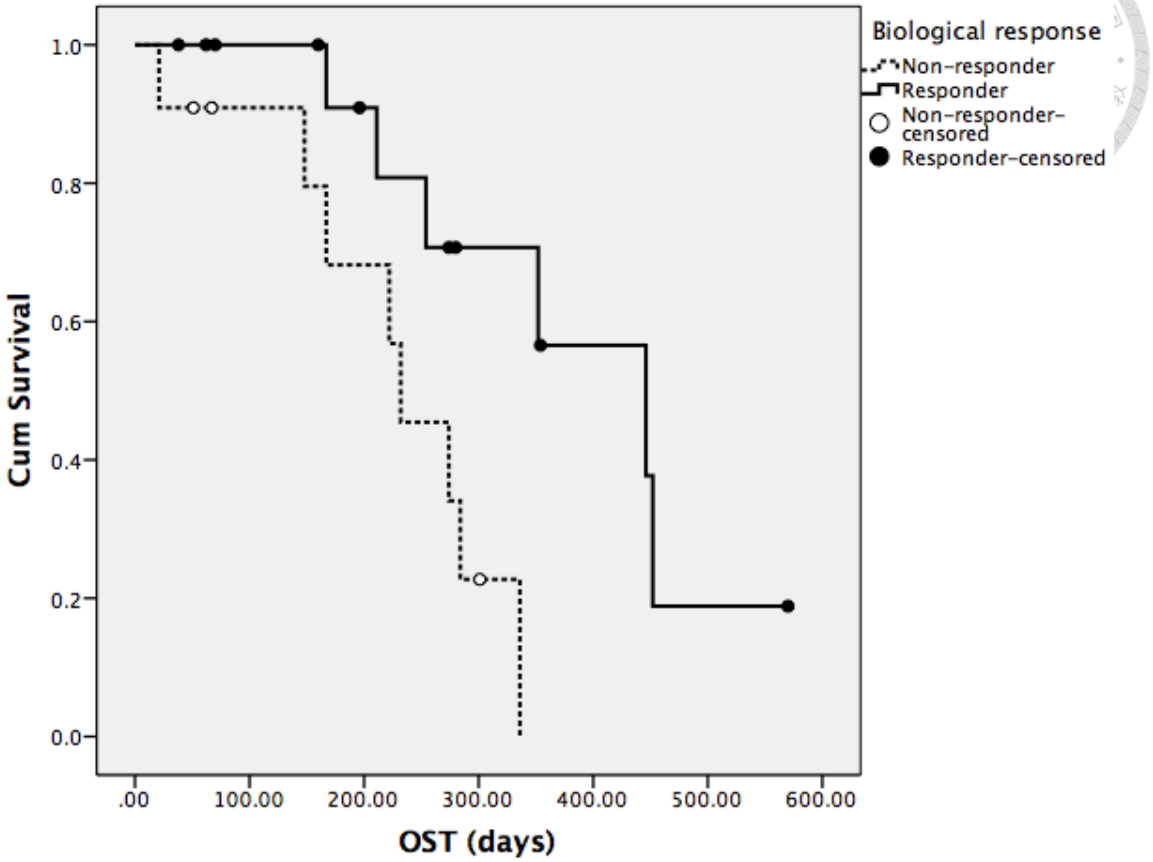

Figure 7. The Kaplan-Meier curve of overall survival time (OST) among different biological responses.

The median OST was 446 days for responders (solid line, n =15) and 232 days for non-responders (dashed line, n =11). (*P* =0.012)

Tables

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Table 1. Comparison of characteristics for vinorelbine and doxorubicin.

Agent	Vinorelbine	Doxorubicin
Classification	Semi-synthetic vinca alkaloid	Anthracyclines
Mechanism	• Inhibiting polymerization of tubulin dimers into microtubules and results in mitotic arrest	• Intercalation and alkylation of DNA • Inhibition the function of RNA and DNA polymerases, topoisomerase II and thioredoxin reductase
Organ of metabolism	Liver	Liver
Organs of elimination	Biliary (major) Renal (minor)	Renal (major) Biliary (minor)
Dosage	Cats: 11.5 mg/m^2 Dogs: 15 mg/m^2	Cats: 20-25 mg/m^2 Dogs: 30 mg/m^2 or 1 mg/kg for dogs smaller than 15 kg
Dosing interval	Weekly for 4 times and then biweekly for 4 times	Usually every 2-3 weeks
Route	IV over 5-10 minutes	IV infusion over 30 minutes
Indications	• Canine pulmonary carcinoma • Had been used in canine TCC, and MCT • Human NSCLC, breast cancer, non-Hodgkin lymphoma	• Canine LSA, MGT, STS • Feline mammary carcinoma, LSA
Adverse events	• Myelosuppression • Extravasation vesicant (mild) • GI toxicity (mild)	• Myelosuppression • Extravasation vesicant • GI toxicity • Hypersensitivity • Cumulative dose-related cardiotoxicity and nephrotoxicity

IV, intravenous; TCC, transitional cell carcinoma; MCT, mast cell tumor; NSCLC, non-small-cell lung cancer; LSA, lymphoma; MGT, mammary gland tumor; STS, soft tissue sarcoma; GI, gastrointestinal

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Table 2. Modified World Health Organization staging system for feline mammary carcinoma.

T: tumor size	
T1	<2 cm maximum diameter
T2	2-3 cm maximum diameter
T3	>3 cm maximum diameter
N: regional lymph node	
N0	No evidence of metastasis
N1	Evidence of metastasis
M: distant metastasis	
M0	No evidence of distant metastasis
M1	Evidence of distant metastasis
Stage	
1	T1, N0, M0
2	T2, N0, M0
3	T3, N0-1, M0; Any T, N1, M0
4	Any T, Any N, M1

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Table 3. Veterinary cooperative oncology group - common terminology criteria for adverse events version 1.1.

Adverse events	Grade				
	1	2	3	4	5
Anorexia	Coaxing or dietary change required to maintain appetite	Oral intake altered (≤3 days) without significant weight loss; oral nutritional supplements/appetite stimulants may be indicated	Of >3 days duration; associated with significant weight loss (≥10%) or malnutrition; IV fluids, tube feeding or force feeding indicated	Life-threatening consequences; TPN indicated; >5 days duration	Death
Vomiting	<3 episode in 24 h, medical intervention not indicated	3 – 10 episodes in 24 h; <5 episodes/day or ≤48 h; parenteral fluids (IV or SC) indicated ≤48 h; medications indicated	Multiple episodes >48 h and IV fluids or PPN/TPN indicated >48 h	Life-threatening (e.g. haemodynamic collapse)	Death
Diarrhea	Increase of up to 2 stools per day over baseline; no increase in frequency, however, consistency decreased over baseline	Increase of 3–6 stools per day over baseline; medications indicated; parenteral (IV or SC) fluids indicated ≤48 h; not interfering with ADL	Increase of >6 stools per day over baseline; incontinence >48 h; IV fluids >48 h; hospitalization; interfering with ADL	Life-threatening (e.g. haemodynamic collapse)	Death
Neutropenia	1500 μ/L to <LLN	1000–1499 μ/L	500–999 μ/L	<500 μ/L	Death

LLN, lower limit of normal; IV, intravenous; SC, subcutaneous; TP, total parental nutrition; PPN, partial parental nutrition; ADL, activities of daily living (eating, sleeping, defecating and urinating)

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Table 4. Comparison of characteristics between two groups.

	VRL group	DOX group	*P* value
Age (years)			0.878
Median (range)	12 (6-14)	12 (7-15)	
Body weight (kg)			0.388
Median (range)	4.06 (2.66-4.86)	3.78 (2.56-5.82)	
Breed			1.000
Mixed	5 (71.4%)	12 (63.2%)	
Persian	1 (14.3%)	4 (21.1%)	
Others	1 (14.3%)	3 (15.8%)	
Gender			0.186
Female intact	1 (14.3%)	8 (42.1%)	
Female neutered	6 (85.7%)	11 (57.9%)	

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Table 5. Comparison of tumor demographics between two groups.

	VRL group	DOX group	*P* value
Gross disease when received chemotherapy			0.973
No	3 (42.9%)	8 (42.1%)	
Yes	4 (57.1%)	11 (57.9%)	
Tumor type [a]			0.787
Adenocarcinoma	3 (60.0%)	10 (66.7%)	
Carcinoma	2 (40.0%)	5 (33.3%)	
Location			
Unilateral	5 (71.4%)	11 (57.9%)	0.529
Bilateral	2 (28.6%)	8 (42.1%)	
Ulceration			0.143
No	4 (57.1%)	5 (26.3%)	
Yes	3 (42.9%)	14 (73.7%)	
Surgical margin [b]			0.510
Clean	2 (50.0%)	6 (66.7%)	
Dirty	2 (50.0%)	3 (33.3%)	
Lymphatic/vascular invasion [b]			0.203
No	2 (50.0%)	1 (11.1%)	
Yes	2 (50.0%)	8 (88.9%)	
Grade of tumor [c]			0.902
1	1 (20.0%)	2 (28.6%)	
2	2 (40.0%)	2 (28.6%)	
3	2 (40.0%)	3 (42.9%)	

a. Two cats in VRL group and 4 cats in DOX group were lack of histopathological diagnosis.
b. Three cats in VRL group and 10 cats in DOX group were lack of information about surgical margin and lymphatic and vascular invasion.
c. Two cats in VRL group and 12 cats in DOX group were lack of tumor grade.

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Table 6. Comparison of clinical stage between two groups.

	VRL group	DOX group	*P* value
Tumor size [a]			0.776
T1	1 (14.3%)	5 (27.8%)	
T2	1 (14.3%)	2 (11.1%)	
T3	5 (71.4%)	11 (61.1%)	
Lymph node [b]			0.028
N0	4 (66.7%)	1 (9.1%)	
N1	2 (33.3%)	10 (90.9%)	
Distant metastasis			0.390
M0	3 (42.8%)	9 (47.4%)	
M1	4 (57.1%)	10 (52.6%)	
Pleural effusion			0.790
No	6 (85.7%)	17 (89.5%)	
Yes	1 (14.3%)	2 (10.5%)	
Stage [c]			0.247
1	2 (28.6%)	1 (5.9%)	
2	0 (0%)	0 (0%)	
3	1 (14.3%)	6 (35.3%)	
4	4 (57.1%)	10 (58.8%)	
Received rescue chemotherapy			0.146
No	4 (57.1%)	16 (84.2%)	
Yes	3 (42.9%)	3 (15.8%)	

a. One cat in DOX group were lack of information about tumor size.
b. One cat in VRL group and 8 cats in DOX group were lack of information about status of lymph node.
c. Two cats in DOX group had not sufficient information to be accurately staged.

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Table 7. Total numbers and dosage of vinorelbine given in VRL group.

Dosage (mg/m^2) of vinorelbine	Total Number of Doses Given
7.36	2
7.50	2
8.00	6
8.25	1
8.28	2
8.50	1
9.30	1
9.70	1
10.00	1
10.30	1
10.35	9
10.40	5
11.40	1
11.50	10
12.60	1

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Table 8. Total numbers of doses and cumulative dosage of doxorubicin in DOX group.

Dosage (mg/m^2) of doxorubicin	Total Number of Doses Given	Cumulative dosage (mg/m^2) of doxorubicin	Total number of Cases
20.0	10	20.0	1
22.2	1	24.7	1
22.3	1	25.0	4
23.5	1	40.0	2
24.7	1	45.0	3
25.0	26	45.7	1
		50.0	2
		70.0	1
		75.0	1
		95.0	1
		97.3	1
		100.0	1

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Table 9. Response to treatment in two groups.

Measurable disease	VRL group	DOX group	*P* value
Objective response rate			0.058
Complete remission	1 (25.0%)	0 (0%)	
Partial remission	2 (50.0%)	2 (18.2%)	
Stable disease	1 (25.0%)	2 (18.2%)	
Progressive disease	0 (0%)	7 (63.6%)	
Non-measurable disease			
Response			0.077
Non-CR/non-PD [a]	3 (100.0%)	4 (50.0%)	
Progressive disease	0 (0%)	4 (50.0%)	
All patients			
Biological response rate			0.008
Biological response [b]	7 (100.0%)	8 (42.1%)	
Progressive disease	0 (0%)	11 (57.9%)	

a. The cases maintained with non-measurable disease at the beginning and the end of treatment.

b. Biological response rate was defined as the total number of cases had been experienced complete remission, partial remission, stable disease and Non-CR/non-PD divided by the number of cases treated.

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Table 10. Summary of median time to progression (TTP) and median overall survival time (OST) for two groups.

	VRL group	DOX group	*P* value
Median TTP	115 days	102 days	0.949
Median OST	352 days	284 days	0.948

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Table 11. Toxicities of treatment in two groups.

	VRL group [a] episodes (%)	DOX group [b] episodes (%)	*P* value
Vomiting	8 (18.2%)	19 (47.5%)	0.004
Grade 1	8 (18.2%)	16 (40.0%)	
Grade 2	0 (0%)	3 (7.5%)	
Grade 3	0 (0%)	0 (0%)	
Anorexia	5 (11.4%)	19 (47.5%)	0.000
Grade 1	3 (6.8%)	5 (12.5%)	
Grade 2	2 (11.4%)	13 (32.5%)	0.001
Grade 3	0 (0%)	1 (2.5%)	
Diarrhea			0.598
Grade 1	3 (6.8%)	4 (10%)	
Neutropenia	19 (43.2%)	0 (0%)	0.000
Grade 1	6 (13.6%)	0 (0%)	
Grade 2	5 (11.4%)	0 (0%)	
Grade 3	6 (13.6%)	0 (0%)	
Grade 4	2 (4.5%)	0 (0%)	

a. Forty-four doses of vinorelbine were administered in VRL group.

b. Forty doses of doxorubicin were administered in DOX group.

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Table 12. Univariate analysis of time to progression (TTP) for factors about patient's demographics.

Factor	n	Median TTP (days)	*P* value
Body weight (kg)			0.411
<3.87	13	39	
>3.87	13	119	
Age (years)			0.504
<12	12	92	
≥12	14	116	
Neuter status			0.021
Intact female	9	119	
Neutered female	17	63	
Gross tumor			0.141
Yes	15	39	
No	11	116	
T stage [a]			0.416
1	6	39	
2	3	102	
3	16	115	
N stage [b]			0.742
N0	5	119	
N1	12	102	
M stage			0.150
M0	11	116	
M1	15	63	
Pleural effusion			0.666
No	23	102	
Yes	3	115	
Stage [c]			0.314
1	3	166	
3	7	102	
4	14	37	
Surgical type [d]			0.758
Regional	9	115	
Chain	14	116	

a. One cat was lack of information of tumor size.
b. Nine cats were lack of information of status of lymph node.
c. Two cats cannot be staged.
d. Three cats did not undergo surgery.

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Table 13. Univariate analysis of time to progression (TTP) for factors about tumor features and response of treatment.

Factor	n	Median TTP (days)	*P* value
Tumor location			0.702
Unilateral	16	115	
Bilateral	10	102	
Ulceration			0.080
No	17	119	
Yes	9	37	
Tumor subtype [a]			0.271
Cribriform	3	119	
Tubulopapillary	9	92	
Combination	4	136	
Grade of tumor [b]			0.857
1	3	63	
2	4	102	
3	5	115	
Lymphatic/vascular invasion [c]			0.929
No	3	63	
Yes	10	102	
Response			0.008
Biological responder [d]	15	119	
Non-biological responder	11	28	

a. Ten cats were lack of information of subtype.
b. Fourteen cats were lack of information about tumor grade.
c. Thirteen cats were lack of information about lymphatic and vascular invasion.
d. Biological response rate was defined as the total number of cases had been experienced complete remission, partial remission, stable disease and Non-CR/non-PD divided by the number of cases treated.

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Table 14. Multivariate analysis of possible prognostic factors for time to progression.

Factor	n	Hazards ratio	95% CI	*P* value
Gross tumor		0.699	0.101-4.835	0.717
No	11			
Yes	15			
Neuter status		5.377	1.406-20.562	0.014
Intact female	9			
Neutered female	17			
Ulceration		1.189	0.466-3.035	0.717
No	17			
Yes	9			
M stage		8.730	0.868-87.811	0.066
M0	11			
M1	15			
Biological response		19.397	3.680-102.249	0.000
Responder [a]	15			
Non-responder	11			

a. Biological response rate was defined as the total number of cases had been experienced complete remission, partial remission, stable disease and Non-CR/non-PD divided by the number of cases treated.

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Table 15. Univariate analysis of overall survival time (OST) for factors about patient's characteristics.

Factor	n	Median OST (days)	*P* value
Body weight (kg)			0.151
<3.87	13	352	
>3.87	13	254	
Age (years)			0.152
<12	12	254	
≥12	14	352	
Neuter status			0.498
Intact female	9	336	
Neutered female	17	284	
Gross tumor			0.408
Yes	15	284	
No	11	336	
Surgical type [a]			0.416
Regional	9	284	
Chain	14	336	
T stage [b]			0.994
1	6	284	
2	3	274	
3	16	336	
N stage [c]			0.166
N0	5	446	
N1	12	254	
M stage			0.344
M0	11	336	
M1	15	284	
Pleural effusion			0.609
No	23	284	
Yes	3	352	
Stage [d]			0.425
1	3	NR	
3	7	254	
4	14	284	
Received rescue chemotherapy			0.159
No	20	336	
Yes	6	222	

a. Three cats did not undergo surgery.
b. One cat was lack of information of tumor size.
c. Nine cats were lack of information of status of lymph node.
d. Two cats cannot be staged.

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Table 16. Univariate analysis of overall survival time (OST) for factors about tumor features and response of treatment.

Factor	n	Median OST (days)	*P* value
Tumor subtype [a]			0.412
Cribriform	3	352	
Tubulopapillary	9	274	
Combination	4	284	
Grade of tumor [b]			0.271
1	3	446	
2	4	254	
3	5	232	
Lymphatic/vascular invasion [c]			0.400
No	3	446	
Yes	10	284	
Tumor location			0.069
Unilateral	16	446	
Bilateral	10	254	
Ulceration			0.196
No	17	336	
Yes	9	232	
Response			0.012
Biological responder [d]	15	446	
Non-biological responder	11	232	

a. Ten cats were lack of information of subtype.
b. Fourteen cats were lack of information about tumor grade.
c. Thirteen cats were lack of information about lymphatic and vascular invasion.
d. Biological response rate was defined as the total number of cases had been experienced complete remission, partial remission, stable disease and Non-CR/non-PD divided by the number of cases treated.

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Table 17. Multivariate analysis of possible prognostic factors for overall survival time.

Factor	n	Hazards ratio	95% CI	*P* value
Body weight (kg)		2.057	0.279-15.140	0.479
<3.87	13			
>3.87	13			
Age (years)		3.002	0.442-20.381	0.261
≥12	14			
<12	12			
Location		1.472	0.197-10.985	0.706
Unilateral	16			
Bilateral	10			
Received rescue chemotherapy		1.013	0.193-5.333	0.987
No	20			
Yes	6			
Ulceration		5.192	1.137-23.706	0.034
No	17			
Yes	9			
Biological response		4.027	1.004-16.152	0.049
Responder [a]	15			
Non-responder	11			

a. Biological response rate was defined as the total number of cases had been experienced complete remission, partial remission, stable disease and Non-CR/non-PD divided by the number of cases treated.

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