Review Article

Review of the Treatment of Canine Cutaneous Mast Cell Tumors

Dank, G.

Koret School of Veterinary Medicine, Hebrew University.

Corresponding author: Dr. Gillian Dank, DVM, D-ACVIM (Oncology), D-ECVIM-Ca (Oncology), Koret School of Veterinary Medicine, Hebrew University.

ABSTRACT

The most common skin tumor in dogs is the mast cell tumor, with an incidence of close to 20% in the canine population. The behavior and progression of MCT are highly heterogeneous, and range from relatively benign to extremely aggressive tumors. There are many treatment options for dogs with mast cell tumors, including surgery, radiation therapy and chemotherapy. Although surgical excision with or without adjuvant radiation may cure most patients with low grade mast cell tumors, there are additional options for dogs with aggressive high grade tumors. The aim of this article is to review the current literature for treatment options for canine cutaneous mast cell tumors.

REVIEW

Mast cell tumors (MCT) are the second most common malignant tumor in dogs, and the most common canine cutaneous tumor (1, 2). The behavior and progression of MCT are highly heterogeneous. Some MCT are behaviorally benign, develop slowly, and persist for years without increasing in size. Whereas other exhibit aggressive growth and progress rapidly to a fatal metastatic disease (2). Treatment options include surgery, radiation therapy and chemotherapy, or a combination of modalities. The treatment should be based on the clinical features, clinical stage and grade (3). The aim of this article is to review the current literature for the treatment options of canine cutaneous mast cell tumors.

Surgery

Surgery is the treatment of choice in localized, non-metastatic canine MCT (3). If complete margins are achieved with surgical excision and there is no evidence of metastasis, surgery is considered curative and no further treatment is required for all grade 1 tumors and most grade 2 tumors (4, 5). Historically, the recommended margins were 3 cm in all directions, with at least one fascial plane on the deep margin. More recent studies have shown that a lateral margin of 2 cm and a deep margin of one fascial plane is sufficient for most grade 1 and 2 MCTs (6). Murphy *et al.* reported 1 year survival rates of 100%, 92% and 46% for grades 1, 2 and 3 respectively (6). Grade 3 tumors should be excised with lateral margins of at least 3 centimeters, plus the deep fascial plane. After resection of grade 2 MCTs, local regrowth rates vary from 0%-27% have been reported for both completely and incompletely excised tumors (5, 7-9). Weisse et *al.,* reported effective local control in 89% of cases in Grade 2 MCT, with a median survival time of 791 days (4).

In cases when the first surgery has not achieved local control, a second surgery with wider margins around the original surgical scar is recommended if feasible. Kry *et al.* compared survival times and local recurrence in dogs with close or incomplete margins treated with primary re-excision or radiation compared to no additional therapy (10). The median survival times for the groups with either a second surgery or radiation therapy were significantly longer that those that received no additional treatment (2930 and 2194 vs. 710 days) (10). Local recurrence was reported in 13% of the re-excision group, 8% of the radiation groups and 38% of the control group. In addition, time to local recurrence was significantly longer in the treatment groups (10).

Based on these publications, the goal of successful treatment in dogs with grade 1 and most grade 2 mast cell tumors should be adequate local control with surgery, which leads to a median survival of over 2000 days, 1 year survival of 92-100%, and very low chances of local recurrence (0-27%) (4, 6, 10).

Radiation

When MCT are incompletely excised, and a second surgery if not feasible, radiation therapy is recommended in order to provide adequate local control.

One of the earlier studies on radiation for incompletely resected canine MCT was published in 1998 by LaDue *et al.* This retrospective study evaluated 56 dogs with macroscopic and microscopic disease. The median disease free interval for dogs with macroscopic disease (21/56 dogs, 38%) was 12 months, compared to 54 months for dogs with microscopic disease (35/56 dogs, 62%) (11). In addition, dogs with tumors >10 cm^3 had shorter disease free intervals than dogs with tumors <9 cm^3 (11). They concluded that radiation therapy should be avoided as a sole therapy in cases of macroscopic disease, due to the risk of radiation induced mast cell granulation, serious systemic effects, and that larger tumors are more radioresistant, and that cytoreductive surgery should be performed in dogs with measurable MCT (11). Radiation therapy was an effective adjuvant therapy to achieve local control of incompletely excised MCT (microscopic disease) and to treat local or regional metastasis (11).

Many additional studies have examined the effectiveness of radiation therapy in dogs with MCT, in cases of incompletely resected tumors, grades 1, 2 and 3 and lymph node metastasis (10-13). There is a controversy regarding the treatment of dogs with adjuvant radiation therapy, as some believe that it is difficult to interpret the added effect, as the recurrence rate is low with surgery alone, even in the case of incomplete histological margins (3). On the other hand, Kry *et al.* showed that there was a significant improvement in survival, duration of local control and percent of local recurrence when radiation was performed after incomplete or close resection compared to dogs that received no additional treatment (10).

When evaluating adjuvant radiation in 45 dogs with incompletely excised, stage 0, grade 2 MCTs, Poirier *et al.* found that only 3 dogs had local recurrence, 2 developed metastasis and 14 developed a second cutaneous tumor (12). They concluded that the adjunctive radiation therapy protocol was well tolerated and efficacious (12). An additional study evaluated the efficacy of radiation in 31 dogs with grade 3 MCTs that were incompletely resected, and found that the median duration of remission was 27.7 months, and the median survival was 28 months (13). They concluded that without further treatment, these tumors had high local regional recurrence rate, and that radiation may effectively be used to manage them (13).

In conclusion, the need for radiation therapy in grade 1 and most grade 2 MCTs with incomplete margins it is still controversial. However, one study compared dogs that received adjunctive radiation to dogs without additional treatment and found there was a significant improvement in survival, duration of local control and reduced percent of local recurrence (10). Additional, larger, prospective studies are needed in order to reach a better understanding of the benefit. Grade 3 tumors appear to benefit from the addition of radiation therapy, as the chances of local recurrence with incomplete margins are higher (13).

Chemotherapy

Chemotherapy can be used in dogs in three ways.

The first is in the neo-adjuvant setting, to reduce tumor burden prior to surgery. This may improve the likelihood of achieving a complete excision, or enable surgery that was not possible prior to the reduction in the size of the tumor (3). Neo-adjuvant prednisone has been evaluated (14). Stanclift *et al.* treated 49 dogs at two doses, either 1 mg/kg prednisone q 24 hours and 2.2 mg/kg prednisone q 24 hours. In both treatment groups there was a significant reduction in the tumor burden. The overall response rate was 70%. There was no significant difference between the two groups (14). There are no studies accurately evaluating the efficacy of neo-adjuvant chemotherapy.

The second in the adjuvant setting is when chemotherapy is used to treat residual microscopic disease where further surgery is not possible and radiation therapy is not available. Two studies have been done regarding chemotherapy in the adjuvant setting. Davies *et al.* looked at 20 dogs with residual microscopic disease that were treated with vinblastine and prednisolone. Eighteen dogs did not have local recurrence after 1 year (15). The second study by Hosoya *et al.* used CCNU (Lomustine) (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) and prednisone in 12 dogs with grade 2 MCT. None of the dogs developed local recurrence or regional/distant metastasis (16). Both studies should be interpreted with care, as the

recurrence rate in grade 1 and 2 tumors with incomplete margins is low (3).

The third indication is in tumors with a high risk for metastasis, either high grade tumors (or grade 3) and grade 2 tumors with risk factors associated with reduced survival. The purpose of the treatment is either to delay or prevent metastasis or to delay progression of existing metastatic disease, however efficacy studies are lacking (17-19). Many different drugs, used as both single agents and combinations, have been studied. The main drugs used include prednisone, vinblastine, CCNU, and tyrosine kinase inhibitors. However, additional protocols have been reported. Chlorambucil and prednisone resulted in an overall response rate of 38% in 21 dogs, and a median progressive free interval for the 8 responders of 533 days (20). Hydroxyurea was evaluated as a single agent in 46 dogs, with an overall response rate of 28%. Two dogs had a complete remission (256 and 448 days) and 11 dogs had a partial remission for a median duration of 46 days (21).

Paclitaxel is part of the taxane family of microtubule inhibitors, paclitaxel suppresses spindle microtubule dynamics. This results in blockage of metaphase-anaphase transitions, and ultimately inhibition of mitosis and induction of apoptosis (22, 23). Paccal vet (Oasmia Pharmaceuticals) is a a cremophor-free formulation of paclitaxel that has been evaluated in two studies, the first to determine the safety and efficacy and the second to compare it to CCNU (22, 23). In the first study, Rivera *et al.* reported a 59% response rate (either a complete or partial response), with a time to progression of 247 days and concluded that the drug appeared safe and effective (22).

The second study was a prospective multicenter randomized double-blind positive-controlled clinical trial in 252 dogs with nonresectable grade 2 or 3 MCTs. The purpose of the study was to compare the response to paclitaxel and lomustine. They concluded that paclitaxel's activity and safety profile were superior to CCNU (23).

The following is a summary of several studies performed, evaluating vinblastine, CCNU, and tyrosine kinase inhibitors (as single agents and combinations) and their effectiveness in the treatment of canine MCTs.

Vinblastine

Thamm *et al.* evaluated oral prednisone and vinblastine in 41 dogs with MCTs (23 in the adjuvant setting and 18 with measurable disease) (24). Overall response rate in the evaluable dogs with gross disease was 47%, consisting of 5 complete responses and 2 partial responses. Median response duration was 154 days. As adjuvant therapy to incomplete surgical resection, prednisone and vinblastine conferred a 57% 1- and 2-year disease-free rate. The median survival time for the entire patient population was not reached; however, the mean survival time (MST) for dogs with grade III MCT was 331 days, with 45% of dogs alive at 1 and 2 years (24).

Three additional studies examining the use of vinblastine in canine MCTs included two dose escalation studies, an efficacy study and a study evaluating vinblastine for adjuvant therapy in high grade tumors (25-27). The dose escalation studies both concluded that vinblastine may be safe to administer at higher than the traditional 2 mg/m^2 dosage. In addition, Bailey *et al.* concluded that the maximum tolerated dose was 3.5 mg/m^2 (26, 27). The efficacy trial administered vinblastine (2 mg/m^2 or 3.5 mg/m^2) to fifty-one dogs with non-resectable grade 2 or 3 cutaneous MCTs. The primary outcome was to measure a reduction in the tumor size. In the 2 mg/m^2 group, 3 (12%) had a partial response for a median of 77 days. In the 3.5 mg/m^2 group the overall response rate was 27%, one dog (4%) had a complete remission and 6 dogs (23%) had a partial remission for a median of 28 days. They concluded that when used as a single-agent, vinblastine had activity against MCTs in dogs (28). Thamm *et al.* looked at 61 dogs with either grade 3 MCT or grade 2 tumors considered to be at a high risk for metastasis. They were treated with vinblastine and prednisone following surgical excision, with or without radiation therapy. The disease free interval was 1305 days, and the overall survival was not reached. 100% of the high risk grade 2 dogs were alive at 3 years, the overall survival for dogs with grade 3 MCT was 1374 days (25). This study compared favorably with historical data on survival for patients with grade 3 MCT with surgery alone, where 6-27% of the patients were alive at 1 year (29, 30).

CCNU- Lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea)

Rassnick *et al.* evaluated the use of CCNU is the treatment of canine MCTs in 19 dogs with measurable MCT (31). Dogs were treated with CCNU at a dosage of 90 mg/m^2 every 3 weeks. One dog had a durable complete response for 440 days. Seven dogs had a partial response for a median duration of 77 days, 6 had stable disease for a median duration of 78 days. The conclusion was that CCNU should be considered an active agent in the treatment of MCT in dogs (31).

Combinations of Vinblastine and CCNU

Three articles have evaluated the efficacy of the combination of vinblastine and CCNU (17, 18, 32). The first was published in 2009 by Cooper *et al.* to evaluate the efficacy and toxicity of CCNU (mean dose of 59 mg/m^2) and vinblastine (2 mg/m^2) in 56 dogs (32). Treatment was administered every 2 weeks. Thirty seven dogs had macroscopic disease (12 (32%) had grade 2 tumors and 17 (46%) had grade 3 tumors) and 20 had microscopic disease. Eight (40%) had grade 2 tumors and 11 (55%) had grade 3 tumors. A 57% response rate was seen in dogs with macroscopic disease for a median duration of 52 weeks. Dogs with macroscopic disease had a median progression free interval of 30 weeks and a median overall survival time of 35 weeks. Dogs with microscopic disease had a median progression interval of 35 weeks and a median overall survival time of 48 weeks (32). The second was published in 2010 by Rassnick *et al.* (18). The study examined the safety and efficacy of a protocol of alternating CCNU (70 mg/m^2) and vinblastine (3.5 mg/m^2), and prednisone (1-2 mg/ kg). Seventeen dogs had macroscopic disease and 35 microscopic disease (either metastatic or grade 3). The response rate in dogs with non-resectable MCTs was 65%; five achieved a complete response (median, 141 days) and six achieved a partial response (median, 66 days). Overall median progression-free interval in dogs treated in the adjuvant setting was 489 days (18). The third study evaluating the combination of CCNU and vinblastine was performed in dogs with grade 2, stage 2 MCTs treated with adequate local therapy and adjuvant systemic therapy (prednisone, vinblastine (2 mg/m^2) and CCNU (60-80 mg/m^2). The results of this study suggested that, in the presence of loco-regional lymph node metastasis in grade 2 MCT, the use of prednisone, vinblastine and CCNU after adequate local-regional therapy can provide a median survival in excess of 40 months (17).

Tyrosine kinase inhibitors (TKIs)

Receptor tyrosine kinases (RTKs) are an important group of cell surface receptors that trigger cellular activation resulting in cellular proliferation, differentiation and survival when stimulated by their ligands. Normal kinase function is critical to cell growth and differentiation, and dysfunction of several RTKs has been characterized in canine MCTs (33, 34). RTKs have also been implicated in angiogenesis and the process of metastasis (3).

Specific small-molecule TKIs are able to block the activity of receptors by competitive inhibition of ATP binding. Two TKIs are approved by the European Medicine Agency for the use in MCT in dogs - Masitinib (Masivet®, Kinavet® AB Science USA, Short Hills, NJ, USA, AB Science Headquarters, Paris, France) and Toceranib Phosphate (Palladia® Manufactured by: Pfizer Inc, Ascoli, Italy). Distributed by: Zoetis Inc., Kalamazoo, MI). Toceranib Phosphate has been approved for use in the United States.

Masitinib

Three articles have been published to date on the use of Masitinib in dogs with MCT. The first was a double-blind, randomized, placebo controlled phase 3 clinical trial in 202 client owned dogs with recurrent or non-resectable grade 2 or 3 non-metastatic MCTs (2). This study found that the Masitinib increased the overall time to progression from 75 days with the placebo to 118 days. The treatment was found to be more effective when given as first line (increasing the time to progression from 75 to 178 days) and was effective in cases with and without a c-kit mutation (2). In the 145 dogs that received the Masitinib, 42.8% experienced an objective tumor response. The most commonly reported adverse effects were vomiting, diarrhea and neutropenia, and the most severe adverse effect reported was proteinuria. They concluded that masitinib was both safe and effective at delaying tumor progression (2). The second study by Hahn *et al.* evaluated the effectiveness of Masitinib for the treatment of non-resectable MCTs in 132 dogs at 12 and 24 months after onset of treatment (grade 2 or grade 3 MCTs) (35). The dogs received either Masitinib (106 dogs) or a placebo (26 dogs). Masitinib significantly improved the survival rate, compared with the placebo, with 62.1% vs. 36.0% of the dogs alive at 12 months and 39.8% vs. 15.0% of the dogs alive at 24 months, respectively. The median overall survival times were 617 and 322 days, respectively. Complete responses at 24 months were observed in 6 of 67 (9.0%) dogs with non-resectable MCTs treated with masitinib. They concluded that Masitinib significantly increased survival rates at 12 and 24 months in dogs with non-resectable MCTs (35).

The third study, by Smrkovski *et al.*, evaluated the use of Masitinib as a first-line therapy and rescue agent in 26 dogs with metastatic and non-metastatic MCTs (36). The overall response rate was 50% (57% for dogs that received the treatment as first-line therapy and 25% for those that received it

as a rescue therapy), and the median survival time for dogs that responded to treatment was 630 days vs. 137 days for dogs that did not respond. Toxicity was reported in 61.5% of the treated dogs. The majority of the adverse events were mild and self limiting, and included liver toxicity, proteinuria, hematologic toxicity and gastrointestinal toxicity. They concluded that the response to treatment was significantly associated with increased median survival (36).

Toceranib Phosphate

In 2009 London *et al.* published the first trial with Toceranib Phosphate (1). The trial was a multi-center, placebo-controlled, double-blind, randomized study in dogs with recurrent MCT. One hundred and forty nine dogs were included in the trial; 86 were treated with Toceranib Phosphate and 63 with a placebo. The objective response rate in Toceranib Phosphate treated dogs was 37.2% (7 complete responses, 25 partial responses) compared with 7.9% in the placebo group (5 partial responses).

Significantly more placebo-treated dogs showed progressive disease during the 6-week trial compared to the dogs treated with Toceranib Phosphate. The objective response rate among Toceranib Phosphate-treated and placebo-escape dogs was 42.8% (21 complete responses, 41 partial responses). The observed biological response rate was 59.5% and included 16 dogs with stable disease. The presence of a c-kit mutation and the absence of regional lymph node metastasis were significantly associated with objective responses (either a complete or partial response) (1). Among dogs with an objective response (62 dogs) the median time to progression was 18.1 weeks. Dogs with grade 2 tumors had a longer time to progression compared to those with grade 3 tumors. Adverse events were generally manageable with dose modification and/or supportive care. They concluded that Toceranib Phosphate has biological activity against canine MCTs (1).

Toceranib Phosphate in combination protocols

Although many clinical trials evaluating the use of Toceranib Phosphate with other chemotherapy agents in canine MCTs are being performed at this time, only two studies have been published to date. The first was a Phase 1 study dose escalation study to evaluate the combination of Toceranib Phosphate and vinblastine (37). The rational of the study was that by combining drugs with known single-agent activity that lack overlapping dose-limiting toxicities and exert anti-tumor activity through different mechanisms they could improve the clinical outcome. Fourteen dogs were enrolled in the trial. The dose limiting toxicity reported in the study was neutropenia resulting in the maximally tolerated dose for vinblastine being low, so that the study did not support this combination. However, evidence of significant activity (71% objective response) and enhanced myelosuppression suggested additive or synergistic activity (37).

The second study, published in 2015 by Burton *et al.*, evaluated intermittent administration of Toceranib Phosphate combined with cytotoxic chemotherapy (38). The purpose of the study was to try to effectively chemosensitize canine MCT while decreasing cost and adverse effects associated with either agent administered as a monotherapy. They administered Toceranib Phosphate and Lomustine to 47 client owned dogs with measurable MCT. The dose limiting toxicity was neutropenia. The overall response rate was 46% (4 complete responses, 15 partial responses) and the overall median progression-free interval was 53 days with the median overall survival time was 131 days. The progression-free interval for dogs with complete response was not reached, and for dogs with a partial response was 131 days. The authors concluded that combined treatment with pulse-administered Toceranib Phosphate and lomustine was well tolerated and may be a reasonable treatment option for dogs with unresectable or metastatic MCT (38).

An additional trial was performed with Toceranib Phosphate, evaluating the combination with radiation therapy. It was a multicenter, prospective trial of hypofractionated radiation therapy, Toceranib Phosphate and prednisone by Carlsten *et al.* in 17 client owned dogs (39). All dogs received prednisone, omeprazole, diphenhydramine, and Toceranib Phosphate. Radiation therapy consisted of 24 Gy delivered in 3 or 4 fractions. The overall response rate was 76.4%, with 58.8% of dogs achieving a complete response and 17.6% a partial response. The median time to best response was 32 days, and the median progression-free interval was 316 days. The overall median survival time was not reached with a median follow-up of 374 days.

The most common toxicoses were gastrointestinal and hepatic. The response rates and response durations reported were higher than those reported for Toceranib Phosphate as a single-agent treatment for MCT. They concluded that the combination was a viable treatment option for unresectable MCT (39).

In summary, the TKIs show a great deal of promise in

the treatment of canine MCTs. However, severe side effects have been reported, and hopefully the future trials with combination therapies will show fewer side effects with higher remission rates and durations. The example by Burton et *al.* is considered a step in the right direction, as Toceranib Phosphate was given only 3 times in a 21 day cycle, instead of three times a week, with Lomustine. The protocol resulted in response rates and durations similar to the original Toceranib Phosphate protocol.

Combination treatment with surgery, chemotherapy and radiation

Although several studies mentioned above included dogs with microscopic disease, one recent study evaluated adjuvant chemotherapy following both surgery and radiation therapy in dogs with stage 2 MCT (17). Stage 2 was defined as one tumor confirmed to the dermis with regional lymph node involvement. All dogs were treated with adequate local control, including surgery of the primary tumor (with adequate margins) and lymph node excision or incomplete excision of the primary tumor followed by radiation therapy plus surgical excision of the lymph node and/or radiation to the lymph node. All dogs received chemotherapy. Local relapse occurred in 2/21 dogs in the area of the previous surgical resection or radiation (17). Four dogs developed *de novo* cutaneous MCTs. The overall median survival was 1359 days. The results of this study suggest that dogs with stage 2 MCTs can achieve long-term median survivals when treated with adequate local control and systemic chemotherapy (17).

CONCLUSIONS

The aim of this article was to review the current literature for treatment options for canine cutaneous mast cell tumors. It is clear that there are several treatment options, each with its advantages and disadvantages, including cost and toxicity. The most important factor to consider when recommending treatment options is to look at the individual dog, including the tumor behavior, clinical signs, histology report, and decide which (if any) protocol would be most beneficial for that specific dog. Unfortunately, as animal health insurance is not common in Israel, the cost of the treatment (including both the drug and the need for follow up blood work) is very important to the clients. At the time this article was submitted, the costs for both Masitinib and Toceranib Phosphate were extremely high and cost prohibitive for many clients. The purpose in treatment is to choose an appropriate protocol with the least amount of toxicity and most efficacy and that is affordable to the client.

REFERENCES

1. London, C.A., Malpas, P.B., Wood-Follis, S.L., Boucher, J.F., Rusk, A.W., Rosenberg, M.P., Henry, C.J., Mitchener, K.L., Klein, M. K., Hintermeister, J.G., Bergman, P.J., Couto, G.C., Mauldin, G.N. and Michels, G.M.: Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clin. Cancer Res. 15: 3856-3865, 2009.
2. Hahn, K.A., Ogilvie, G., Rusk, T., Devauchelle, P., Leblanc, A., Legendre, A., Powers, B., Leventhal, P.S., Kinet, J.P., Palmerini, F., Dubreuil, P., Moussy, A. and Hermine, O.: Masitinib is safe and effective for the treatment of canine mast cell tumors. J. Vet. Intern. Med. 22: 1301-1309, 2008.
3. Blackwood, L., Murphy, S., Buracco, P., De Vos, J.P., De Fornel-Thibaud, P., Hirschberger, J., Kessler, M., Pastor, J., Ponce, F., Savary-Bataille, K. and Argyle, D.J.: European consensus document on mast cell tumours in dogs and cats. Vet. Comp. Oncol. 10: e1-e29, 2012.
4. Weisse, C., Shofer, F.S. and Sorenmo, K.: Recurrence rates and sites for grade II canine cutaneous mast cell tumors following complete surgical excision. J. Am. Anim. Hosp. Assoc. 38:71-73, 2002.
5. Séguin, B., Leibman, N.F., Bregazzi, V.S., Ogilvie, G.K., Powers, B.E., Dernell, W.S., Fettman, M.J. and Withrow, S.J.: Clinical outcome of dogs with grade-II mast cell tumors treated with surgery alone: 55 cases (1996-1999). J. Am. Vet. Med. Assoc. 218: 1120-1123, 2001.
6. Murphy, S., Sparkes, A.H., Smith, K.C., Blunden, A.S. and Brearley, M.J.: Relationships between the histological grade of cutaneous mast cell tumours in dogs, their survival and the efficacy of surgical resection. Vet. Rec. 154: 743-746, 2004.
7. Séguin, B., Besancon, M.F., McCallan, J.L., Dewe, L.L., Tenwolde, M.C., Wong, E.K. and Kent, M.S.: Recurrence rate, clinical outcome, and cellular proliferation indices as prognostic indicators after incomplete surgical excision of cutaneous grade II mast cell tumors: 28 dogs (1994-2002). J. Vet. Intern. Med. 20: 933-940. 2006.
8. Fulcher, R.P., Ludwig, L.L., Bergman, P.J., Newman, S.J., Simpson, A.M. and Patnaik, A.K.: Evaluation of a two-centimeter lateral surgical margin for excision of grade I and grade II cutaneous mast cell tumors in dogs. J. Am. Vet. Med. Assoc. 228:210-215, 2006.
9. Simpson, A.M., Ludwig, L.L., Newman, S.J., Bergman, P.J., Hottinger, H.A. and Patnaik, A.K.: Evaluation of surgical margins required for complete excision of cutaneous mast cell tumors in dogs. J. Am. Vet. Med. Assoc. 224: 236-240, 2004.
10. Kry, K.L. and Boston, S.E.: Additional local therapy with primary re-excision or radiation therapy improves survival and local control after incomplete or close surgical excision of mast cell tumors in dogs. Vet. Surg. 43: 182-189, 2014.
11. LaDue, T., Price, G.S., Dodge, R., Page, R.L. and Thrall, D.E.: Radiation therapy for incompletely resected canine mast cell tumors. Vet. Radiol. Ultrasound. 39: 57-62, 1998.
12. Poirier, V.J., Adams, W.M., Forrest, L.J., Green, E.M., Dubielzig, R.R. and Vail, D.M.: Radiation therapy for incompletely excised

grade II canine mast cell tumors. J. Am. Anim. Hosp. Assoc. 42: 430-434, 2006.
13. Hahn, K.A., King, G.K. and Carreras, J.K.: Efficacy of radiation therapy for incompletely resected grade-III mast cell tumors in dogs: 31 cases (1987-1998). J. Am. Vet. Med. Assoc. 224: 79-82, 2004.
14. Stanclift, R.M. and Gilson, S.D.: Evaluation of neoadjuvant prednisone administration and surgical excision in treatment of cutaneous mast cell tumors in dogs. J. Am.Vet. Med. Assoc. 232: 53-62, 2008.
15. Davies, D.R., Wyatt, K.M., Jardine, J.E., Robertson, I.D. and Irwin, P.J.: Vinblastine and prednisolone as adjunctive therapy for canine cutaneous mast cell tumors. J. Am. Anim. Hosp. Assoc. 40:124-130, 2004.
16. Hosoya, K., Kisseberth, W.C., Alvarez, F.J., Lara-Garcia, A., Beamer, G., Stromberg, P.C. and Couto, C.G.: Adjuvant CCNU (lomustine) and prednisone chemotherapy for dogs with incompletely excised grade 2 mast cell tumors. J. Am. Anim. Hosp. Assoc. 45: 14-8. 2009.
17. Lejeune, A., Skorupski, K., Frazier, S., Vanhaezebrouck, I., Rebhun, R.B., Reilly, C.M. and Rodriguez, C.O. Jr.: Aggressive local therapy combined with systemic chemotherapy provides long-term control in grade II stage 2 canine mast cell tumour: 21 cases (1999-2012). Vet. Comp. Oncol. 13:267-80, 2015.
18. Rassnick, K.M., Bailey, D.B., Russell, D.S., Flory, A.B., Kiselow, M.A., Intile, J.L., Malone, E.K., Balkman, C.E. and Barnard, S.M.: A phase II study to evaluate the toxicity and efficacy of alternating CCNU and high-dose vinblastine and prednisone (CVP) for treatment of dogs with high-grade, metastatic or nonresectable mast cell tumours. Vet. Comp. Oncol, 8:138-52, 2010.
19. Miller, R.L., Van Lelyveld, S., Warland, J., Dobson, J.M. and Foale, R.D.: A retrospective review of treatment and response of high-risk mast cell tumours in dogs. Vet. Comp. Oncol., doi: 10.1111/vco.12116, 2014.
20. Taylor, F., Gear, R., Hoather, T. and Dobson, J.: Chlorambucil and prednisolone chemotherapy for dogs with inoperable mast cell tumours: 21 cases. J. Small. Anim. Pract. 50: 284-289, 2009.
21. Rassnick, K.M., Al-Sarraf, R., Bailey, D.B., Chretin, J.D., Phillips, B. and Zwhalen, C.H.: Phase II open-label study of single-agent hydroxyurea for treatment of mast cell tumours in dogs. Vet. Comp. Oncol. 8:103-111, 2010.
22. Rivera, P., Akerlund-Denneberg, N., Bergvall, K., Kessler, M., Rowe, A., Willmann, M., Persson, G., Kastengren Fröberg, G., Westberg, S. and von Euler, H.: Clinical efficacy and safety of a water-soluble micellar paclitaxel (Paccal Vet) in canine mastocytomas. J. Small Anim. Pract. 54:20-27, 2013.
23. Vail, D.M., von Euler, H., Rusk, A.W., Barber, L., Clifford, C., Elmslie, R., Fulton, L., Hirschberger, J., Klein, M., London, C., Martano, M., McNiel, E.A., Morris, J.S.,Northrup, N., Phillips, B., Polton, G., Post, G., Rosenberg, M., Ruslander, D., Sahora, A., Siegel, S., Thamm, D., Westberg, S., Winter, J. and Khanna, C.: A randomized trial investigating the efficacy and safety of water soluble micellar paclitaxel (Paccal Vet) for treatment of nonresectable grade 2 or 3 mast cell tumors in dogs. J. Vet. Intern. Med. 26: 598-607, 2012.
24. Thamm, D.H., Mauldin, E.A. and Vail, D.M.: Prednisone and vinblastine chemotherapy for canine mast cell tumor - 41 cases (1992-1997). J. Vet. Intern. Med. 13: 491-497, 1999.
25. Thamm, D.H., Turek, M.M. and Vail, D.M.: Outcome and prognostic factors following adjuvant prednisone/vinblastine chemotherapy for high-risk canine mast cell tumour: 61 cases. J. Vet. Med. Sci. 68: 581-587, 2006.
26. Vickery, K.R., Wilson, H. and Vail, D.M.: Thamm DH. Dose-escalating vinblastine for the treatment of canine mast cell tumour. Vet. Comp. Oncol. 6: 111-119, 2008.
27. Bailey, D.B., Rassnick, K.M., Kristal, O., Chretin, J.D. and Balkman, C.E.: Phase I dose escalation of single-agent vinblastine in dogs. J. Vet. Intern. Med. 22: 1397-1402.
28. Rassnick, K.M., Bailey, D.B., Flory, A.B., Balkman, C.E., Kiselow, M,A., Intile, J.L. and Autio, K.: Efficacy of vinblastine for treatment of canine mast cell tumors. J. Vet. Intern. Med. 22: 1390-1396, 2008.
29. Patnaik, A.K., Ehler,W.J. and MacEwen, E.G.: Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. Vet. Pathol. 21: 469-474, 1984.
30. Bostock, D.E., Crocker, J., Harris, K. and Smith, P.: Nucleolar organiser regions as indicators of post-surgical prognosis in canine spontaneous mast cell tumours. Br. J. Cancer. 59: 915-918, 1989.
31. Rassnick, K.M., Moore, A.S., Williams, L.E., London, C.A. and Kintzer, P,P., Engler, S.J. and Cotter, S.M.: Treatment of canine mast cell tumors with CCNU (lomustine). J. Vet. Intern. Med. 3: 601-605. 1999.
32. Cooper, M., Tsai, X. and Bennett, P.: Combination CCNU and vinblastine chemotherapy for canine mast cell tumours: 57 cases. Vet. Comp. Oncol. 7: 196-206, 2009.
33. Downing, S., Chien, M.B., Kass, P.H., Moore, PE. and London. C.A.: Prevalence and importance of internal tandem duplications in exons 11 and 12 of c-kit in mast cell tumors of dogs. Am. J. Vet. Res. 63: 1718-1723, 2002.
34. London, C.A., Galli, S.J., Yuuki, T., Hu, Z.Q., Helfand, S.C. and Geissler, E.N.: Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. Exp. Hematol. 27:689-697, 1999.
35. Hahn, K.A., Legendre, A.M., Shaw, N.G., Phillips, B., Ogilvie, G.K., Prescott, D.M., Atwater, S.W., Carreras, J.K., Lana, S.E., Ladue, T., Rusk, A., Kinet, J.P., Dubreuil, P., Moussy, A. and Hermine, O.: Evaluation of 12- and 24-month survival rates after treatment with masitinib in dogs with nonresectable mast cell tumors. Am. J. Vet. Res. 71:1354-1361, 2010.
36. Smrkovski, O.A., Essick, L., Rohrbach, B.W. and Legendre. A.M.: Masitinib mesylate for metastatic and non-resectable canine cutaneous mast cell tumours. Vet. Comp. Oncol. 13:314-321, 2015.
37. Robat, C., London, C., Bunting, L., McCartan, L., Stingle, N., Selting, K., Kurzman, I. and Vail, D.M.: Safety evaluation of combination vinblastine and toceranib phosphate (Palladia(R)) in dogs: a phase I dose-finding study. Vet. Comp. Oncol. 10:174-183, 2012.
38. Burton, J.H., Venable, R.O., Vail, D.M., Williams, L.E., Clifford, C.A., Axiak-Bechtel, S.M., Avery, A.C. and Thamm, D.H.: Pulse-Administered Toceranib Phosphate Plus Lomustine for treatment of unresectable mast cell tumors in dogs. J. Vet. Intern. Med. 29: 1098-1104, 2015.
39. Carlsten, K.S., London, C.A., Haney, S., Burnett, R., Avery, A.C. and Thamm, D.H.: Multicenter prospective trial of hypofractionated radiation treatment, toceranib, and prednisone for measurable canine mast cell tumors. J. Vet. Intern. Med. 26: 135-141, 2012.