Demystifying Mast Cell Tumors in Dogs

Douglas H. Thamm, VMD, DACVIM (Oncology)
Colorado State University
Fort Collins, CO

Mast cell tumor (MCT) represents the most common malignant cutaneous tumor in the dog, and is commonly encountered in small animals. There is a large degree of variation in the histologic appearance and biologic behavior of canine MCT, ranging from histologically and behaviorally benign to histologically and behaviorally malignant. However, 65 to 80% of MCT will remain local diseases. Knowledge of the signs associated with worrisome prognosis, and the steps to take to address the potential for recurrence and/or metastasis, can help to simplify the approach to this sometimes frustrating neoplasm. Likewise, newer information regarding local and systemic treatment of MCT has increased the management options available for veterinarians and owners to consider.

Diagnosis

Canine MCT have been referred to as "the great pretender", because they can look and feel like anything. This can include soft, subcutaneous masses that can feel exactly like lipomas. Thus, needle aspiration cytology should be offered for any lump or bump encountered. Cytology is sufficient to achieve a diagnosis of MCT in approximately 90% of dogs. The classic appearance is a population of large round cells with central nuclei and abundant cytoplasm, with characteristic blue-purple cytoplasmic granules. Granules will not be visible in approximately 10% of MCT, which may confound the diagnosis in a small number of cases. It is common to see other inflammatory cells, such as eosinophils and neutrophils, admixed with the MCT cells.

When a presumptive diagnosis of MCT is made, it is useful to perform needle aspiration cytology of the regional lymph node at the same time, whether or not it feels enlarged, to rule out early metastasis.

To stage or not to stage

The majority of canine MCT, while locally aggressive, are unlikely to metastasize. Having an idea of which are likely to behave aggressively prior to surgery may help to identify those patients in which additional staging, to rule out disease elsewhere, should be undertaken preoperatively.

Prior studies have identified several prognostic factors associated with MCT: (1) Histologic grade is one of the strongest -- dogs with high-grade (grade III) tumors may die of their disease rapidly despite appropriate local therapy; (2) Clinical stage - Dogs with metastasis to regional lymph nodes or other structures at presentation have a less favorable long-term prognosis; (3) Location - Tumors in the preputial, perianal, oral, subungual (nail bed) and other mucocutaneous sites classically have worse prognoses; (4) Recurrence following initial surgical excision is a negative prognostic indicator; (5) The presence of systemic signs (anorexia, vomiting, hematemesis, melena) is a strong negative prognostic indicator, as it often indicates systemic dissemination; (6) Recent rapid growth or tumor ulceration are also worrying signs.

Animals with tumors displaying these criteria may have a higher likelihood of metastasis, and thus a thorough search for disease elsewhere is reasonable prior to undertaking definitive therapy. This may also be reasonable in lower-risk patients if very expensive or aggressive treatment is likely to be necessary, or if the tumor is in a location not amenable to wide surgical excision. In the absence of these factors, it is reasonable to proceed immediately to appropriately aggressive surgical excision (See below).

Complete staging for canine MCT should include cytologic evaluation of the regional lymph node, abdominal ultrasound, and thoracic radiographs. Of these tests, abdominal imaging and lymph node cytology are the most likely to yield important results. Cytology of abnormal lymph nodes or organs in the abdomen is indicated, however aspirates of structurally normal liver and spleen are rarely useful. Pulmonary parenchymal involvement from MCT is extremely uncommon, but involvement of thoracic lymph nodes is possible. If radical, expensive or potentially disfiguring surgery is being contemplated, an incisional biopsy may also be considered for histologic grading. The utility of other tests such as bone marrow aspiration cytology and buffy coat smear is questionable at best, and they are not routinely performed in the author's practice.

If no evidence of disease elsewhere is found, appropriate local therapy can be pursued. Identification of disease in the regional lymph node means that this should be removed as well at the time of surgery, and that additional systemic therapy should be considered irrespective of histologic grade. Identification of disease beyond the regional lymph node usually means that surgery will be of little or no benefit.

Surgery for mast cell tumors

Even well-differentiated MCT are associated with aggressive local tissue infiltration. Thus, it is necessary to include a generous margin of normal-appearing tissue on all sides of the tumor (including deep) to insure that any microscopic nests of tumor are removed. The standard recommendation is to remove a minimum of 3 cm of normal-appearing tissue 360 degrees around the tumor, and at least one normal fascial plane deep. The entire specimen should be submitted in one piece, preferably with the margins inked, so that the pathologist can assess all margins for adequacy of excision. There is accumulating information, however, that surgical

margins less than 3 cm may be sufficient in "tight spots". This seems especially true for low/intermediate grade tumors, and those that are fairly small in diameter.

When necessary, very aggressive or radical surgical procedures, such as amputation or body wall resection, are reasonable to consider. Prior to contemplating procedures such as these, complete staging is recommended, and incisional biopsy for determination of histologic grade may be helpful. When dealing with a low or intermediate-grade tumor, very aggressive surgery is reasonable because the likelihood of metastasis is low.

The question of whether or not to administer perioperative histamine blockers is a matter of personal choice. The risk of a serious degranulation reaction is very small unless the tumor is extensively handled (which should not happen if 3 cm of normal tissue is removed!).

Interpreting the pathology report

Two equally important pieces of information need to be gleaned from the pathology report: (1) Histologic grade; and (2) Adequacy of surgical margins. If only a representative piece of the tumor is submitted, margins cannot be evaluated and the utility of the report is cut in half. Pathologists often utilize a numeric grading scheme, where "Grade I" is well-differentiated and "Grade III" is poorly differentiated, however some pathologists will now utilize words such as "low, intermediate or high-grade" or "well, poorly or intermediately differentiated" in place of a numerical scale. If information regarding grade or margins is not provided, it should be requested from the pathologist.

Low or intermediate grade MCT with complete surgical margins usually require no further therapy, as the risk of recurrence or metastasis is only approximately 10%. However, regular rechecks for recurrence, metastasis, or new cutaneous masses is indicated. Low or intermediate grade tumors with incomplete surgical margins have a high chance of recurrence, but a low chance for metastasis. Thus, further aggressive local therapy is reasonable. When possible, immediate re-excision of the surgical scar (and an additional 3 cm tissue in all directions and another fascial plane deep) is the most useful treatment. The entire excised tissue should be inked and re-submitted for histopathology. When this is not possible, the next best option would be the use of radiation therapy. Chemotherapy may be useful to delay or prevent recurrence in cases where additional surgery or radiotherapy is not possible or has been declined.

High grade MCT with complete surgical margins have a low chance for recurrence, but a high chance for eventual metastasis. Systemic therapy (e.g. chemotherapy) can be offered in an attempt to delay or prevent this. High grade MCT with incomplete margins have a high likelihood of both recurrence and metastasis: Therapy designed to address both of these possibilities (e.g. additional surgery or radiotherapy, with chemotherapy) is indicated.

There is new information that assessment of mitotic index (a measure of the rate of proliferation, which can be assessed on any histology slide), may be a strong predictor of outcome, identifying intermediate grade tumors at high risk of spread and, potentially, high-grade tumors at lower risk of spread.

Recently, a 2-tier grading schema for MCT has been proposed, which classifies tumors as either high-grade or low-grade based on mitotic activity, presence of multinucleated cells, bizarre nuclei, and karyomegaly. Postsurgical outcome was substantially different between these 2 grades, and inter-pathologist agreement regarding grade was quite high. Independent validation of these interesting results will likely be required prior to the universal adaption of this new schema.

Special stains and genetic tests

A variety of specialized histochemical or immunohistochemical tests for assessment of proliferation (agyrophilic nucleolar organizer region, or AgNOR staining, Ki67, and proliferating cell nuclear antigen, or PCNA) have been evaluated for their predictive value and have likewise been demonstrated to correlate well with postsurgical outcome. However, it is not clear as yet whether these more cumbersome assessments provide any more prognostic information than that provided by simple assessment of mitotic index.

Recently, expression of KIT, a tyrosine kinase receptor for the hematopoietic growth factor stem cell factor (SCF), has been demonstrated in canine and feline MCT. Several studies have demonstrated, in 20-40% of canine MCT, the presence of mutations in the *c-kit* gene, leading to constitutive activation in the absence of bound SCF. In the multiple studies, MCT possessing *c-kit* gene mutations or altered subcellular localization as assessed by immunohistochemistry (e.g. a shift from the normal membranous location to an intracellular location) are associated with an inferior prognosis when compared to those with wild-type *c-kit* and normal KIT protein localization. Both *c-kit* gene sequencing and KIT protein immunohistochemistry are available through multiple academic laboratories in the U.S. See below for additional information regarding the KIT protein and canine MCT.

Caution owners against a "wait and see" approach

The importance of addressing the potential for local recurrence the very first time the tumor appears cannot be overstated. Owners should be strongly cautioned against adopting a "wait and see" attitude, with the intent of becoming more aggressive if/when the tumor grows back. Recurrent tumors are likely to grow more quickly, invade more deeply, and are more likely to ulcerate or become painful. In a recent study, dogs with MCT that were locally recurrent at the time systemic therapy was started were more than 4 times more likely to die as a result of MCT than dogs that started therapy at the first occurrence.

In addition to aggressive local surgery, several other local therapeutic modalities have been investigated for the adjuvant treatment of canine MCT. Radiation therapy (RT) has proven to be a very effective local treatment modality when combined with "marginal" surgical excision. 2-year control rates of 85 to 90% can be expected when incompletely excised low- or intermediate-grade MCT are treated with RT. Radiation therapy to bulky tumors is consistently less effective than RT to microscopic disease, with a one-year control rate of approximately 50%.

Animals with undifferentiated MCT, MCT that have metastasized, or tumors in a historically unfavorable location (see above) may benefit from the addition of some form of systemic therapy to appropriate local therapy. In addition, aggressive surgery or RT may be cosmetically unappealing or financially impossible for some owners. Recently, several studies have been published investigating various systemic therapies for measurable canine MCT, the results of which are summarized in **Table 1**.

Table 1. Response to medical therapy in measurable canine mast cell tumors

Agent(s)	Number Treated	%CR[a]	%PR[b]	%ORR[c]	Median Resp. Duration
Prednisone	25	4%	16%	20%	NR[d]
Vincristine	27	0%	7%	7%	NR
CCNU (Lomustine)	21	6%	38%	44%	79 d[e]
Pred/Vinblastine	17	33%	13%	47%	154 d
P/C/V[f]	11	18%	45%	63%	74 d
COP-HU[g]	17	23%	35%	59%	53 d
Pred/VBL/CCNU	37	24%	32%	57%	52 wks
LDI-100	46	14%	14%	29%	NR
Calcitriol	10	10%	30%	40%	74-90 d
Hydroxyurea	46	4%	24%	28%	46 d (for PRs)
Pred/Chlorambucil	21	14%	24%	38%	533 d

[a] CR = Complete response

[b] PR = Partial response

[c] ORR = Overall response rate

[d] NR = Not reported

[e] Excludes patient that experienced a CR, euthanized without evidence of disease after 440 days

[f] P/C/V = prednisone/cyclophosphamide/vinblastine

[g] COP-HU = cyclophosphamide/vincristine/prednisone/hydroxyurea

Systematic evaluation of postoperative treatment for MCT at high risk of metastasis remains understudied. Historically, this "high-risk" designation includes high-grade/anaplastic MCT, MCT arising from mucous membranes, and MCT with regional metastasis. Recent evidence suggests that MCT with high indices of proliferation, as assessed via immunohistochemical means or assessment of mitotic index, may be candidates for postoperative chemotherapy as well, irrespective of grade.

Three studies have been published evaluating the efficacy of chemotherapy with prednisone and vinblastine (VBL) in the prevention of recurrence or metastasis in the post-surgical setting.

Prednisone and VBL administration

Prednisone is administered orally at an initial dose of 2 mg/kg daily, for the first week, and this dose is tapered and discontinued over approximately 3 months. VBL is given as a rapid intravenous bolus at 2 mg/m2 every 1-2 weeks. The standard postoperative protocol consists of weekly injections for 4 weeks, followed by 4 biweekly injections.

Adverse effects

Adverse effects are noted in approximately 20% of patients, usually after the first dose of VBL. These are mild in most. Mild adverse effects include self-limiting vomiting, neutropenia without evidence of sepsis (7-day neutrophil count less than 1,000/µL), or lethargy/soft stool. Severe adverse effects occur in only approximately 5% of patients.

Efficacy

Although randomized, placebo-controlled clinical trials are lacking, there is accumulating evidence from single-arm studies that adjuvant therapy for MCT at high risk for metastasis can improve patient outcome. In one study, 27 dogs with incompletely or marginally resected MCT, mostly of intermediate grade, were treated with prednisolone and VBL chemotherapy. Only one dog (3.7%) experienced local recurrence, and four (15%) developed another cutaneous MCT. A second study evaluated the use of postoperative prednisone and VBL for dogs with MCT considered to be at high risk for metastasis (node-positive, mucous membrane origin, or high histologic grade). In this study, dogs with high grade MCT had a median survival time of 1374 days. A third study reported 70% 1- and 2-year disease-free survival percentages following prednisone/VBL in high-grade MCT.

Interestingly, one study suggested a profound difference between the outcome of a high-grade tumor and an intermediate-grade tumor with lymph node metastasis. Despite the presence of lymph node metastasis, 90% of patients with grade II tumors with positive lymph nodes were disease-free at one year. Patients with grade III tumors treated in the adjuvant setting had 2-year survival rates of 60%. This appears to be a significant improvement over historical data employing surgery alone, which report a median survival of 8 months and a 2-year survival percentage of less than 15%.

There is information suggesting that many dogs may tolerate doses of VBL in excess of 2 mg/m^2. It remains to be seen if dose-escalation of VBL will translate into improved efficacy. Several investigators have reported on the efficacy of postoperative therapy with other drugs e.g. prednisone/lomustine, VBL/cyclophosphamide/prednisone, or VBL/lomustine/prednisone for postoperative MCT therapy, also with encouraging results.

Other clinicians recommend postoperative treatment with lomustine (CCNU), an oral alkylating agent, or alternating lomustine and vinblastine.

New directions

Perhaps the most important recent finding with potential to translate into new and exciting forms of therapy is the discovery that the majority of canine (and human) mast cell neoplasms express the tyrosine kinase growth factor receptor KIT, and a large minority of canine MCT possess a mutation in the gene coding for the KIT protein. This gene codes for a transmembrane protein that serves as the receptor for the growth factor stem cell factor, important in the maturation of normal mast cells and other hematopoietic cells. Mutations can render KIT active even in the absence of bound stem cell factor. In other words, these mutations mean that the cells are receiving signals to proliferate and survive when they normally would not, leading to unchecked growth. New molecules have been developed that inhibit signaling through the KIT tyrosine kinase, these compounds are able to interfere with the proliferation of canine MCT in vitro. Furthermore, evidence of antitumor activity has been documented in dogs with MCT treated with these compounds. The 2 veterinary-approved molecules in this class are toceranib (Palladia®, Pfizer) and masitinib (Masivet®/Kinavet®, AB Science).

Following encouraging *in vitro* and early-phase clinical studies with toceranib, a multi-center, placebo-controlled, double-blind, randomized study of toceranib was performed in dogs with recurrent or metastatic grade II or III MCT. Dogs were randomized to receive oral toceranib 3.25 mg/kg or placebo every other day for 6 weeks in the blinded phase. Thereafter, eligible dogs received open-label toceranib. The overall response rate in toceranib-treated dogs (n = 86) was 37.2% (7 complete response, 25 partial response) versus 7.9% (5 partial response) in placebo-treated dogs. Among the toceranib treated responders, the median duration of objective response and time to tumor progression was 12.0 weeks and 18.1 weeks, respectively. Interestingly, dogs whose MCT harbored activating mutations in the *c-kit* gene were roughly twice as likely to respond to toceranib than those with wild-type *c-kit* (60% vs 30%). The efficacy observed in this study led to the full approval of toceranib by the U.S. FDA.

Subsequent to FDA approval, considerable clinical experience with toceranib has been amassed by U.S. oncologists. Important observations in this post-approval phase include the high incidence of gastrointestinal toxicity in dogs treated with the label-indicated dosage and schedule: most U.S. oncologists currently utilize a dose of 2.75 mg/kg every-other-day or Monday-Wednesday-Friday, which appears well tolerated by the majority of dogs. Gastrointestinal toxicity, in the form of inappetance, weight loss, diarrhea, and occasionally vomiting or melena, are the most common adverse effects, and are generally manageable with symptomatic therapy, drug holidays and dosage reductions as necessary. Other adverse effects reported include mild to moderate leucopenia, and occasional muscle pain or leukotrichia.

A clinical trial of similar design was recently completed with masitinib in dogs with recurrent or unresectable MCT. Masitinib was administered at a dose of 12.5 mg/kg daily. This study demonstrated significantly improved time to progression in masitinib-treated versus placebo-treated dogs, and again, response rate and outcome was improved in dogs with MCT harboring *c-kit* mutations. Gastrointestinal adverse effects (vomiting or diarrhea) were most common but were mild in the vast majority of cases (usually grade 1 or 2) and self-limiting. Myelosuppression can also occur, particularly neutropenia, although in most cases this is mild. A small percentage of dogs developed a protein-losing nephropathy leading to edema. Increases in urea and creatinine were seen in some dogs, although this was not clear if it was drug-related or not – however, caution is recommended when using this drug in patients with pre-existing renal disease. Hemolytic anemia was also seen as a rare occurrence in the study.

Practical monitoring of patients on RTKIs

- Clinicians and owners should be vigilant to monitor patients for adverse effects, as described above. Monitoring of complete blood counts, serum biochemistry profiles and urinalysis is suggested at baseline, after 2 weeks of therapy and monthly thereafter. Dose reductions, drug holidays or supportive care, in the form of gut protectants, may be indicated.

A variety of unanswered questions exist regarding the use of RTK inhibitors such as toceranib and masitinib for the treatment of animal cancer. These include: (1) Can they be given to cats safely? The answer to this appears to be yes, but evidence of efficacy is limited to a small number of cats that have been treated with imatinib. (2) Are there other tumors where they might be efficacious? Anecdotal or early evidence of efficacy has been reported for toceranib for the treatment of diverse diseases including multiple myeloma, soft-tissue sarcoma, metastatic osteosarcoma and carcinomas of the mammary gland, thyroid and anal sac; similarly, there are reports of antitumor responses to masitinib in dogs with T-cell lymphoma. In these other tumor types, efficacy may be as a result of the targeting of other RTKs important in cancer, such as PDGFR or VEGFR2. (3) Are they effective for the postoperative treatment

of incompletely resected or “high-risk” MCT? This has not been studied. Further complicating this question, it is not at all clear how long these drugs should be continued in the postoperative setting. (4) Can they be used together with chemotherapy or radiation therapy? This is not clear, however preliminary evidence suggests that toceranib can be combined safely with coarsely fractionated (palliative) radiation therapy, with encouraging antitumor activity. Early reports suggest additive toxicity when toceranib is combined with traditional cytotoxic chemotherapeutic agents such as VBL or CCNU, which suggest that significant dosage reductions of the cytotoxic agent may be necessary if the drugs are to be safely combined. Early abstracts suggest that masitinib/chemotherapy combinations (doxorubicin/carboplatin) may be tolerated at chemotherapy doses approaching the maximum tolerated dose. There is also interest in investigating the combination of RTKIs and low dose continuous (“metronomic”) chemotherapy.

Summary

While recent advances in the medical treatment of MCT are very exciting, it is important to remember that, aggressive surgery remains the mainstay of treatment for canine MCT, and is sufficient to successfully treat the majority of MCT encountered in practice.

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