INTRODUCTION

In dogs, mast cell tumours (MCTs) are most commonly found in the cutaneous tissue; and this is the most common malignancy of the skin in dogs. In most dogs, tumours are solitary, but in about 10–15%, the tumours are multiple. Tumours usually occur in older dogs (mean age 9 years) with no sex predilection. Boxers, Rhodesian ridgebacks, pugs, Boston terriers, pit-bull terriers and Weimaraners are at high risk (4 to 8 times more than the general population) for developing MCTs. Shar-Peis, particularly young dogs, are predisposed to developing MCT, and these tumours are often poorly differentiated and biologically aggressive.

It is uncommon to diagnose MCTs without skin involvement in dogs. Mast cell tumours vary greatly in appearance, and no estimate of their malignancy or prediction of their behavior can be made on clinical appearance alone. Some MCTs may be present for months to years before rapidly disseminating; others act aggressively from the beginning. Occasionally, mechanical manipulation during examination of this tumour causes degranulation of mast cells, producing erythema and wheal formations. This phenomenon has been observed in both dogs and cats (the "Darier's sign") and is considered of diagnostic significance. Owners may report that the tumour enlarges rapidly and then diminishes in size over a period of about 24 hours. Such a history should increase the clinician's suspicion of mast cell tumour.

The clinical appearance of MCTs in dogs may vary widely but diagnosis is relatively easy using aspiration cytology. Presurgical aspiration of these tumours provides a cytology specimen characterized by round cells that may have well-stained and large cytoplasmic granules (well-differentiated) or that may be more anaplastic with small, poorly staining cytoplasmic granules. Eosinophils are often seen in aspirates of MCTs because of eosinophil chemotaxis to histamine release.

GRADING

While diagnosis of MCT often can be made by fine needle aspiration cytology, excisional biopsy is required for accurate histologic grading of the tumour. Histopathologic grading of the tumour has been correlated with both recurrence and survival, with Patnaik grade 1 MCT essentially having benign behaviour, and grade 3 being more likely to show malignant behaviour. Grade 2 MCT have an "intermediate" behaviour.\(^1\)

One study found that mitotic index was strongly prognostic for survival with grade 2 MCT with a mitotic index (per 10 high power fields) of < or = 5 having a survival time of 70 months, compared to 5 months if the mitotic index was > 5.\(^2\) Another study looking at mitotic index suggested 3 levels of prognostic significance, with dogs that had tumours with mitotic index < 1 having very long survivals; with mitotic index 1–7 having median survival of 18 months; and dogs with mitotic index > 7 having median survival of 3 months. Our
clinical experience has been that we have occasionally seen some MCT behave aggressively when the mitotic index was less than 5. Usually these have a mitotic index of 2 or more; but we have seen lymph node metastasis with MCT with a mitotic index of 0 or 1.

A recent pathology reevaluation of the grading system for MCT proposed a new two-tiered scheme that identified dogs at high risk regardless of their Patnaik grade. In that study, a Kiupel high-grade MCT was characterized by at least 7 mitotic figures in 10 hpf; or at least 3 multinucleated (3 or more nuclei) cells in 10 hpf; or at least 3 bizarre nuclei (highly atypical with marked indentations, segmentation, and irregular shape) in 10 hpf; or karyomegaly (nuclear diameters of at least 10% of neoplastic mast cells vary by at least two-fold). One advantage in this system is its simplicity for pathologists in applying, eliminating some of the subjectivity inherent in the Patnaik system. The study showed a median survival time of < 4 months for high-grade tumours and > 2 years for low-grade tumours. The report showed that all dogs with Kiupel high-grade MCT had a uniformly negative outcome with their MCT; however, not all dogs with low-grade tumours had uniformly positive outcomes. The difficulties in relying on the Kiupel system are that it is less informative for dogs with low-grade tumours than the Patnaik system, and that few clinical/therapeutic data have been published using this system - the majority is based on the Patnaik system and mitotic index. Therefore, clinically, while the Kiupel system is being used more and more, we still use the Patnaik system plus mitotic index to a large extent, and it is very helpful when the pathologist provides all 3 pieces, as recently suggested by the VCS Oncology-Pathology Working Group on MCT.

**STAGING**

All dogs with MCTs should be staged to determine the extent of their disease. This is especially important for dogs being considered for aggressive surgery such as amputation, or radiation therapy.

To establish the stage of a dog with a cutaneous MCT, the following information is needed:

1. **Complete blood count (CBC), serum chemistry profile and urinalysis**
2. **Lymph node aspirates**
   The clinician should perform fine-needle aspiration of the regional lymph node if the node is enlarged. The presence of clusters of mast cells (and eosinophils) is an indication that the MCT may no longer be confined to the primary site. Mast cells may also infiltrate a regional lymph node in a dog with a MCT as an inflammatory response to the tumour; therefore a suspicious cytology result should be confirmed by biopsy.
3. **Radiographs and ultrasonography**
   Splenomegaly or hepatomegaly may indicate spread of MCTs systemically. Pulmonary metastasis of MCTs is rare. Ultrasonography is most useful for staging a dog with MCT if used in conjunction with histopathology or cytology.
4. **Bone marrow aspirates**
   The presence of > 1% mast cells indicates systemic spread of the neoplasm. In a recent report
4.5% of dogs with cutaneous MCT had either > 1% or abnormal mast cells in a bone marrow aspirate.

5. **Miscellaneous tests**

Faecal occult blood tests may be useful in evaluating patients with mast cell disease. In many cases, faeces may contain small amounts of blood that are insufficient to produce melena. Evidence of gastrointestinal bleeding in a patient with a MCT should prompt the clinician to treat with medications that block the effects of mast cell hyperhistaminaemia (i.e., H₂ blockers, such as famotidine).

6. **Buffy coat smears**

Care must be exercised in interpreting buffy coat smears, because mastocythaemia has been reported in a variety of canine acute inflammatory diseases. For this reason, most oncologists now feel that this test has limited applicability to staging of dogs with MCT.

**PROGNOSTIC FACTORS**

Recent clinical research has led to the identification of prognostic factors for dogs undergoing therapy with surgery and radiation therapy, and this same research has led to changes in thinking about other previously well-accepted prognostic factors.

**Major Prognostic Factors**

* **Tumour Grade**

Recent studies using aggressive surgical technique, and histology to examine margins (rather than the surgeon's clinical impression), have shown that dogs with grade 2 MCT have a lower rate of local recurrence and longer survival rates than was previously believed. Nonetheless, grading is still an important prognostic factor. Grade 3 tumours are more likely to be incompletely excised, more likely to metastasize, and nearly 4 times more likely to result in death than tumours of lower grades.

* **Mitotic Index**

As discussed above, mitotic index is clearly an important prognostic factor, regardless of which grading system is used. Depending on the study, different cutoffs for the mitotic index predict different outcomes, but in all studies and systems, the lower the mitotic index the better. Mitotic index is always counted in the area of the tumour that has the highest mitotic activity and is always reported as number of mitoses per 10 high power fields (hpf).

* **Surgical Margins**

The completeness of excision (i.e., whether the surgical margins are "dirty") is an important prognostic factor and also important in determining if further surgery or adjunctive radiation therapy is needed. There is often disparity between the surgeon's expectation of margins and those assessed by histopathology. In one study, 22 of 59 tumours thought to have been excised widely had either questionable (10) or incomplete (12) excision based on histological examination. However, not all incompletely resected MCT will recur.
Nonetheless, dogs with incomplete excisions are more likely to develop metastatic disease. Because of this finding, and because tumour recurrence is more common following incomplete excision, the clinician is counseled to obtain clear surgical margins wherever possible, and not to rely on a marginal excision.

**Tumour Stage**

Although it is strictly considered stage 3, the presence of multiple MCTs does not necessarily confer a worse prognosis. Dogs with lymph node metastases are nearly 8 times as likely to die of MCT as those without. As noted above, the presence of small numbers of mast cells on cytology of a lymph node is suspicious for metastasis but not conclusive; so this is an indication for biopsy for confirmation. A potential problem with staging is that small numbers of mast cells may be found in the circulation, spleen, liver, and bone marrow, so the significance of such a finding is unclear. In one study, the presence of small numbers of mast cells in these locations did not seem to influence survival in dogs with grade 2 MCTs.

**Minor Prognostic Factors**

**c-kit (CD117) Expression Pattern**

All MCT express c-kit but the pattern of expression within cells has been found to be prognostic, with 3 different patterns identified. Pattern 1 (perimembrane) staining is favourable, with only 2.4% of 42 dogs dying of MCT; pattern 3 (diffuse cytoplasmic) staining is unfavourable, with 38.5% of 13 dogs dying of MCT; and pattern 2 (focal cytoplasmic) staining is intermediate, with 25.6% of 43 dogs dying of MCT. It should be noted that staining pattern is not the same as c-kit mutation status, so it is not predictive of response to tyrosine kinase inhibitors (e.g., toceranib, masitinib).

**Ki-67 Expression**

Ki-67 is a proliferative index that has been shown to have some prognostic value. In Patnaik grade 2 tumours with < 1.8% positivity, 77 to 95% of dogs lived 3 years; whereas dogs with tumours that had > 1.8% positivity had 3-year survival of 21–33%.

**TREATMENT**

Control of canine MCTs involves the use of surgery, chemotherapy, or radiation therapy, either individually or in combination.

**Surgery**

Surgical excision is indicated if the tumour is solitary and evidence of lymph node involvement or systemic spread is lacking. Excision should be wide and deep to a minimum margin of 2–3 cm around the perceived borders of the tumour and one fascial plane below. With this approach, recurrence of grade 1 and grade 2 MCTs is very low. One study examined the completeness of surgical excision at margins 1 cm, 2 cm and 3 cm from the edges of grade 1 and 2 MCT. All grade 1 tumours were excised 1 cm from the tumour borders, while only 75% of grade 2 tumours were completely excised at the same distance. Surgical excision should be aggressive. All excised tissue should be examined histologically for completeness of tumour excision. Extension of the tumour beyond the surgical borders or a report of "close"
margins should prompt wider excision if this is possible. A second excision should include the previous excision site plus lateral margins of 2 cm and additional deep tissue. If the tumour cannot be completely excised due to tumour location or other factors, or if it is a grade 3 MCT, further therapy is indicated. The dog should be evaluated for radiation therapy, if available. Chemotherapy should be considered if staging disclosed metastases, or if the MCT is Patnaik grade 3, Kiupel high grade, or if the mitotic index is high (see above).

**Radiation Therapy**

Mast cell tumours are quite sensitive to the effects of radiation therapy, even at moderate doses. Dogs that have no measurable evidence of disease after surgical removal of MCTs, but which had incomplete excision on histologic examination of excised tissues have significantly longer tumour control and survival following radiation therapy than other dogs. Thus, post-surgical radiation therapy for incompletely excised tumours seems beneficial. Mast cell tumours of the extremities often present the greatest challenge for complete surgical excision. For well- or moderately differentiated tumours in these locations, combined modalities of less aggressive surgical "debulking" followed by radiation therapy may be a more acceptable treatment - both functionally and cosmetically.

Palliative radiation therapy may relieve symptoms of extensive or systemic disease. When the tumour is poorly differentiated or metastasis is already confirmed, high-dose intermittent radiation treatments may improve the quality of life by stopping bleeding or reducing the size of a bulky or irritating tumour. In these cases, a fully fractionated course would be costly and reduce the amount of time spent by owners with the dog. A short, coarsely fractionated series of treatments may provide relief from symptoms, although it will not increase life span. Systemic therapy, as outlined later, should also be considered.

**Systemic Therapy**

Metastatic disease occurs more frequently in dogs that had Patnaik grade 3/Kiupel high-grade MCT, in dogs with MCT with high mitotic index, and in dogs that had incomplete surgical excision of their cutaneous tumour. When MCTs have metastasized or spread systemically, localized therapies, such as surgery or radiation, are appropriate only as palliation of discomfort or mechanical obstruction. For these dogs, systemic therapy is required.

**Corticosteroids** are primarily palliative, but some long-term responses do occur. Oral prednisone is given as long as the tumour does not progress. Anecdotally, dogs that are tumour-free after six months have a lower incidence of recurrence; therefore, therapy is usually discontinued at this time.

**Vinblastine** and prednisolone were used to treat MCTs in one study. The response rate in dogs with measurable disease was 47%; there were 5 complete responses and 2 partial responses. The median response duration was 5 months (1 to > 22 months). Dogs with lower-grade tumours seemed to respond better. Vinblastine is myelosuppressive, and a CBC should be performed weekly and prior to administering the drug.
CCNU (lomustine) was given to 19 dogs. One had a complete response for 15 months, and 7 had a partial response for an average of 3 months (1 to 9 months) (42% overall response rate). Another study, using a lower dosage level reported a lower response rate of 1%. CCNU can be combined with prednisolone. Concurrent SAMe has been shown to reduce the risk and severity of hepatic toxicity. If using CCNU, monitor CBC (especially platelet count), creatinine and liver enzymes, and discontinue if thrombocytopenia or increased liver enzymes or creatinine occur.

Vinblastine and CCNU in an alternating combination protocol have also been evaluated. In one study, a 57% response rate was seen in dogs with macroscopic disease. Dogs with macroscopic disease had a median progression-free survival time (PFST) of 30 weeks and a median overall survival time (OST) of 35 weeks. Dogs with microscopic disease had a median PFST of 35 weeks and a median OST of 48 weeks. In another study, response rate in dogs with nonresectable MCTs was 65%. Overall median progression-free survival (PFS) time in dogs treated in the adjuvant setting was 16 months.

Vincristine was found to be an inactive agent for the treatment of MCT and often causes severe gastrointestinal toxicity. It is not recommended as a first-line chemotherapy agent for the treatment of MCT.

Mutations in the proto-oncogene tyrosine kinase receptor c-kit were shown to lead to constitutive phosphorylation of the gene product and believed to be important in the development and progression of at least some canine MCT. There is no evidence that such mutations are breed associated. Recently, there has been an increasing interest in c-kit mutation status due to the release of tyrosine kinase inhibitors (TKIs) specifically for use in veterinary patients. Two TKIs have undergone registrational studies in dogs with mast cell tumours.

Masitinib (Masivet/Kinavet; AB Science) primarily targets c-kit. A placebo-controlled randomized double blind phase III clinical trial in 202 dogs with non-metastatic grade 2 or 3 MCTs, showed a 12-month complete response (CR) rate of 11.1% (vs. 2.9% with placebo [not significantly different]) and "controlled disease" (overall response rate [ORR] plus stable disease [SD]) of 23.1% (vs. 5.9% with placebo [significantly different]). Dogs with Kit mutations had 27.3% CR (vs. 0% with placebo [not significant]) and 31.8% "controlled disease" (vs. 0% with placebo [not significant]). The study did report significantly prolonged time to progression (TTP) of 118 days (vs. 75 days with placebo), which was greater in dogs with Kit mutations (230 days vs. 42 days with placebo). A more recent study reported improved survival in dogs with nonresectable MCTs compared with results for the placebo, with 59 of 95 (62.1%) and 9 of 25 (36.0%) dogs alive at 12 months and 33 of 83 (39.8%) and 3 of 20 (15.0%) dogs alive at 24 months, respectively; and median overall survival times of 20.5 months and 10.7 months. Masivet is approved in Europe and the UK for dogs with recurrent or nonresectable grade 2 or 3 MCT regardless of c-kit mutation status, and as Kinavet in the US.

Toceranib (Palladia; Pfizer Animal Health) is similar to sunitinib, a human TKI, in that it is active against several members of the split-kinase RTK family (VEGFR, PDGFR and Kit), and has both anti-angiogenic and
anti-tumour activity. In a trial in 57 dogs with various cancers (carcinomas, sarcomas, MCTs, melanomas, and lymphomas), the overall response (complete response plus partial response) rate was 28%. In a placebo-controlled randomized study in dogs with nonresectable grade 2 and 3 MCTs, Palladia-treated dogs had an overall response rate (ORR, complete response [CR] plus partial response [PR]) of 37.2% (7 CR, 25 PR) compared to 7.9% (5 PR) with placebo (n = 63). Following placebo escape, the total ORR for all 145 dogs receiving Palladia was 42.8% (21 CR, 41 PR); dogs with Kit mutations had an ORR of 60% (vs. 31.3% in dogs without mutations). Dogs without lymph node metastasis had higher response rate (48% vs. 33% in dogs with metastasis). Grade 3 or 4 adverse events were seen in 20.7% of the dogs treated with toceranib. Combination protocols of Palladia with other chemotherapy drugs are being developed.

**Palliation of Paraneoplastic Symptoms**

Ancillary drug therapy is important with canine MCTs.

Animals with mastocytosis or any bulky mast cell disease should receive H₂ antagonists, as rapid degranulation of neoplastic mast cells may follow surgery or chemotherapy. Elevated systemic histamine levels may also be seen with recurrent disease. The objective of the therapy is to prevent gastrointestinal ulceration associated with elevated levels of histamine and to treat ulcers already present. This is most likely to occur in dogs with larger, bulky disease, with recurrence of cutaneous disease, or with systemic spread of MCT. H₂ antagonists such as famotidine reduce gastric acid production by competitive inhibition of the action of histamine on H₂ receptors of the gastric parietal cells. Omeprazole, which inhibits gastric acid production by the gastric parietal cells through proton pump inhibition, may also be used. Dogs with clinical evidence of gastrointestinal ulceration and bleeding may benefit from sucralfate therapy. Sucralfate reacts with stomach acid to form a highly condensed, viscous, adherent, paste-like substance that binds to the surface of both gastric and duodenal ulcers. The barrier formed protects the ulcer from potential ulcerogenic properties of pepsin, acid, and bile, allowing the ulcer to heal.

H₁ antagonists should be considered for use in addition, before and after surgical removal of canine MCTs to help prevent the negative effects of local histamine release on fibroplasia and wound healing. Loratadine has been shown to be very effective at inhibiting histamine release by blocking degranulation from normal canine mast cells, and therefore may be a good choice for palliation of dogs with MCT.

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