Introduction

Mast cell tumors (MCT) are one of the most common cutaneous neoplasms in dogs (7 to 21% depending on the study). They are generally easy to diagnose cytologically and histologically, and rarely are poorly differentiated enough to require additional staining (e.g. T-blue, Giemsa, C-kit, Tryptase) to confirm a diagnosis. Figure 1 shows a well differentiated MCT compared to a poorly differentiated MCT (Fig. 2). Despite their often straightforward diagnosis, their biologic behavior is more complicated to determine as each can vary from benign to highly aggressive, potentially fatally malignant. Furthermore, no single factor, including histologic grade, is consistently reliable enough on its own to predict behavior, mortality, and need for/response to multimodal therapy. Rather, information gathered from the clinical presentation, staging, histologic grading systems, cell proliferation analysis, KIT protein expression patterns and c-kit gene proto-oncogene mutations are collectively utilized to allow treatment decisions to be made for each individual tumor.

This handout provides a review of current histologic grading systems, clinical features and molecular tests that are useful for determining which tumors are at high risk for aggressive behavior.

Histologic Grading Systems and Survivability

According to the classical Patnaik grading system (Patnaik 1984), grade 1 tumors are well-differentiated and confined to the dermis. Grade 2 tumors involve the dermis and hypodermis, and are well-differentiated to intermediately differentiated with 0-2 mitoses per high power field (HPF). Grade 3 tumors are poorly differentiated with 3-6 mitoses per HPF. Survival times in dogs with cutaneous mast cell tumors excised with wide surgical margins and no additional follow up treatment were calculated (Patnaik 1984). Of the 83 dogs in this study, 93% of the 30 dogs with grade 1 tumors survived beyond the 1500 day period. 47% of the 36 dogs with grade 2 tumors survived beyond 1500 days. Only one of the 17 dogs with a grade 3 tumor (6%) survived beyond 1500 days. Unfortunately, this study did not include whether death was due to MCT-related disease.
**Histologic Grading Systems and Survivability** (continued)

Survivability was assessed in 137 dogs with surgically resected mast cell tumors over a 12 month period with no follow up treatment (Sabattini 2015). The survival probability at 1 year was 100% for the 18 dogs with grade 1 tumors, 87% for the 83 dogs with grade 2 tumors, and 16% for the 36 dogs with grade 3 tumors. Median survival for grade 3 was 108 days.

The Kiupel grading system (Kiupel 2011) consists of only two tiers where tumors with at least 7 mitotic figures, 3 multinucleated cells, 3 bizarre nuclei or 10% karyomegaly of cells in ten HPFs are classified as high-grade, rather than low-grade. The median survival time is <4 months for high grade MCT and >2 years for low-grade MCT. The survival probability at 1 year (based on Sabattini 2015) was 95% for the 89 dogs with low-grade tumors and 24% for the 48 dogs with high-grade tumors. Median survival time for high-grade tumors was 150 days.

When evaluating both systems together (Sabattini 2015), the survival probability at 12 months was 94% for the grade 2/low-grade category and 46% for the grade 2/high-grade. Median survival time for grade 2/high-grade was 698 days.

**Metastatic Risk**

In Kiupel 2011, 90% of dogs with high-grade MCTs died of MCT-associated disease and 70% developed metastasis, while only 5% of low-grade MCTs died due to MCT associated disease. Another retrospective study (Stefanello 2015) evaluating metastatic rates in 386 dogs with previously untreated cutaneous MCTs showed an overall metastatic rate of 18.7%. 5.8% of dogs with grade 1 tumors had metastasis. 16.5% of dogs with grade 2 tumors showed metastasis. Of these grade 2 tumors, 83% were a Kiupel low-grade and 16.5% of these metastasized. The remaining 17% were Kiupel high-grade, and 14.6% metastasized. 48.8% of dogs with grade 3 tumors showed metastasis. 14.9% of Kiupel low-grade tumors showed metastasis and 30.8% of Kiupel high-grade tumors showed metastasis. Thus, this study shows that substantial proportions of dogs with grade 2 (16.5%) and grade 1 (5.8%) tumors and dogs with low-grade tumors (14.9%) have metastasis.

As an aside, the Stefanello study also found that tumor diameter greater than or equal to 3 cm and tumor ulceration were clinical variables significantly associated with nodal metastasis at the time of initial examination. However, additional prognostic parameters are still needed to create an appropriate treatment plan.

**Cellular Proliferation**

Cell proliferation reflects the cells in the cell cycle and their rate through it. Mitotic index only reflects cells in the M-phase of the cycle, and 30% of aggressive MCTs have a low mitotic index (Kiupel 2011). Ki-67 identifies cells in all phases of the cell cycle, and AgNOR scores reflect the rate at which cells progress thorough it. Thus, the Ki67 x AgNOR product gives a more accurate reflection of cellular proliferation than the M-phase index (i.e. mitotic count) alone. According to a study of 56 dogs with one mast cell tumor treated with surgical excision only (Webster 2007), 60% of dogs with Ki67 x AgNOR scores >54 died of MCT related disease within 12 months of diagnosis.
KIT protein expression and c-kit prot-onco gene mutations:

C-kit gene is involved in mast cell survival, differentiation, migration and proliferation. Abnormal expression patterns of KIT protein (as identified by IHC labeling patterns 2 and 3) and tumors with activating mutations in the c-kit gene (as screened for by PCR) have been associated with shorter overall survival time due to their mast cell disease and increased incidence of local recurrence in comparison to those with KIT pattern 1 and without activating mutations (Webster 2006 and 2004), but respond well to tyrosine kinase inhibitor drugs. The incidence of c-kit gene mutations is likely to be between 9% and 15% in all canine MCTs, and as many as 50% for high-grade MCTs.

Subcutaneous MCT

A specific grading system is not currently available for subcutaneous MCTs. They generally have an indolent clinical course and a 94% survival probability at 12 months (Sabattini 2015), although there is a subset of them that behave more aggressively. Therefore, evaluation of specific histologic criteria and molecular markers utilized for cutaneous MCTs is still recommended for subcutaneous MCTs.

Conclusions and Clinical Recommendations

Surgical margin recommendations, staging and chemotherapeutic protocols based on the aforementioned tumor grades and prognostication results are beyond the scope of this handout but are nicely reviewed in an article provided by Sledge DG, et al. See reference on page 4. The decision diagram published in his article (see page 4) is a useful aid in determining if systemic therapy should be considered.

In summary, each cutaneous mast cell tumors should to be handled in a case by case fashion, and good communication between the submitting veterinarian, the pathologist and the veterinary oncologist is important.

Phoenix Lab provides a MCT prognostic panel as a send-out test that includes the proliferation markers Ki67 and AgNOR, C-KIT immunohistochemistry and PCR testing for c-kit mutations in exons 8 and 11. Please call our lab for current pricing, turn-around times or additional information.
Considerations for inclusion of systemic therapy in treatment of dogs with canine cutaneous mast cell tumors

Grading

High grade

Low grade

Low grade AND Negative staging

Positive staging

Staging

Evaluate proliferation indices

PCR for activating c-Kit mutation in exon 11 and IHC for KIT

High proliferation indices

Low proliferation indices AND No c-Kit mutation AND KIT pattern 1

Demonstration of c-Kit mutation OR KIT pattern 2 or 3

No indication for further treatment

Systemic therapy should be considered


References:


Stefanello D, et al. Comparison of 2- and 3- category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009-2014). JAVMA 2015; 246(7): 765-769

