INTRODUCTION

Canine mast cell tumors (MCT) are one of the most common tumors in veterinary oncology. They also are one of the most frustrating to treat. The biologic behavior of these tumors varies widely. Many have a benign behavior and are cured with surgery alone. However, some mast cell tumors are malignant. They can be very locally invasive, they can metastasize, and they can be fatal. Therefore, when treating canine MCTs it is important to be appropriately aggressive, ensuring that patients are neither subjected to unnecessary treatments nor deprived of needed therapy. There are several prognostic factors used to guide therapy decisions, including a newly proposed grading scheme and prognostic panel. This talk will discuss how these and other prognostic factors should be used to help guide treatment decisions.

DIAGNOSTIC EVALUATION FOR DOGS WITH MAST CELL TUMORS

Staging Diagnostics

Initial staging diagnostics should include CBC, chemistry panel, and urinalysis. When MCTs metastasize, regional lymph nodes are affected most commonly, followed by liver and spleen, and occasionally the bone marrow. The regional lymph node should be assessed either with cytology or preferably histopathology (by surgically removing the lymph node at the same time as the primary tumor). Abdominal ultrasound is advised prior to surgery. Aspirates of the liver, spleen, or other intra-abdominal organs should be performed only if they appear abnormal on imaging or metastasis is strongly suspected; routine aspiration of organs is not recommended. The author recommends thoracic radiographs, particularly in geriatric patients, as part of a pre-anesthetic screen. However, pulmonary metastasis is rare. Bone marrow cytology usually is reserved for patients with high grade tumors (especially if aggressive local therapy, such as radiation therapy, is going to be pursued) or patients with disseminated disease. Buffy coat profiles are neither sensitive nor specific, and are not recommended.

Mast Cell Tumor Histopathology and Tumor Grade

The grading scheme first proposed by Patnaik divided MCTs into three groups based on cellular morphology, mitotic index, and depth of invasion. The grading scheme strongly correlated with survival after surgery alone: 93% of dogs with grade I tumors were alive 1500 days after surgery, compared to 44% of dogs with grade II tumors and 6% of dogs with grade III tumors. However, this grading system has lost much of its clinical utility. In the original study published by Patnaik, of 83 MCTs, 30 (36%) were classified as grade I, 36 (43%) as grade II, and 20 (17%) as grade III. Current the vast majority of canine MCTs are reported as grade II. Additionally, grading criteria have been modified and can vary significantly among pathologists. In a study where 10 pathologists evaluated and graded the same 60 MCTs, there were only 4 tumors where all pathologists agreed on grade. Additionally 6 tumors received grades of I, II, and III by different pathologists. Recently, a new 2-tier grading system was proposed. Using these new guidelines, MCT are considered high-grade if they meet any of the following criteria:
≥7 mitoses in 10 high-powered fields, ≥3 multinucleated cells (3 or more nuclei) in 10 hpf, ≥3 bizarre nuclei in 10 hpf, karyomegaly. With this new grading system, dogs with low-grade MCT treated with surgery alone had a median survival of >2 years, compared to 4 months for high-grade MCT. It is essential that clinical veterinarians familiarize themselves with the components of these MCT grading systems and to ensure that the microscopic description is in agreement with the grade assigned to the tumor.

**Mast Cell Tumor Prognostic Panels**

MCT prognostic panels are becoming more widely available. The parameters assessed in these panels include Ki-67 and proliferating cell nuclear antigen (PCNA) immunostaining, argyrophilic nucleolar organizing region (AgNOR) histochemical staining, Kit (CD117) immunostaining, and Kit PCR to screen for common mutations in the juxtamembrane domain. High Ki-67 and AGNOR scores have been associated with local tumor recurrence, MCT metastasis, and MCT-associated death. The prognostic significance of PCNA is less clear. One study showed that dogs that died from their MCT had higher PCNA counts than dogs that did not, but there was great overlap between groups. Other studies have not demonstrated any prognostic significance for PCNA counts. Because of these results, most commercially available panels have dropped this marker. Immunostaining for the receptor tyrosine kinase (RTK) Kit can identify abnormal localization of the protein within the cytoplasm instead of the cell membrane. Increase localization of Kit in the cytoplasm is correlated with higher Patnaik tumor grade and higher Ki-67 score, although the impact on clinical outcome has not been reported. PCR can be used to screen for internal tandem duplications (ITD) in the negative regulatory juxtamembrane domain (most commonly in exons 8 or 11). These mutations result in constitutive activation of Kit in the absence of ligand binding, and are associated with increased risk of local recurrence, increased risk of metastasis, and higher tumor proliferation indices.

There are a few important caveats with prognostic panels. First, not every lab offers all of the tests listed above. It is important to know exactly what is included before ordering a prognostic panel. Second, proliferation indices (Ki-67, PCNA, and AgNOR) can be scored using a variety of systems that can yield substantially different cut-off values that define a favorable or unfavorable prognosis. It is important to know the cut-off values for the specific lab you are using, and you should ensure that those values have been validated. Third, it can easily take 1-3 weeks to get the full prognostic panel results. For many MCT, this additional time will not impact prognosis, but for advanced stage or rapidly growing tumors the results might not be timely enough.

Recommendations regarding when to order a MCT prognostic panel are still being refined. While we are still learning, it is not wrong to recommend ordering a full panel for all MCT. However, as we gain more experience, particularly with cases where there is discordance within the panel or between the panel and histologic grade, this recommendation might change. Others currently are recommending the MCT panel primarily for all low-grade MCT in an effort to identify those occasional tumors that have favorable features on routine histopathology but a more aggressive phenotype. At a minimum, the panel should be considered for patients where there is disagreement among other prognostic factors (e.g. a very small, well-circumscribed dermal tumor that on histopathology has no abnormal morphologic features but a high mitotic index).
TREATMENT OPTIONS

Surgery

For dogs with no evidence of metastasis, wide surgical excision is the treatment of choice whenever possible. Historically, recommendations have been made to remove MCTs with lateral margins of 3 cm and a deep margin of 1 fascial plane. However, >90% of low grade MCT (grades I and II using the earlier Patnaik system) are completely excised with a lateral margin of 2 cm and a deep margin of 1 fascial plane. For larger or more invasive MCTs, though, wider surgical margins should still be attempted. Similarly, for tumors with substantial peritumoral edema secondary to mast cell degranulation, it can be difficult to visually define the extent of the tumor at surgery. A wider margin is also recommended for these tumors to help ensure complete excision.

If a MCT is classified as low grade, has a favorable prognostic panel, and is completely excised with clean margins, no further treatment is indicated. Continued monitoring is always recommended, though. The author recommends a physical examination 1, 2, 4, 6, 9, and 12 months after surgery, and then every 6 months thereafter. If a low-grade MCT is incompletely excised, historically adjuvant radiation therapy has been recommended to improve local control (see below). However, radiation probably is not necessary for all dogs in this category. Reported local recurrence rates are only 18-30%. Since some of the parameters in the MCT prognostic panel have been linked to local recurrence and/or metastasis, perhaps these results can be used to decide whether adjuvant radiation is indicated or whether careful monitoring might be appropriate.

For MCT that are high grade and/or that have unfavorable prognostic panel results, surgery is still indicated if the disease is localized. However, due to the increased risk for metastasis, adjuvant chemotherapy is recommended (see below).

Radiation Therapy

Radiation therapy is most effective when combined with surgery. Definitive protocols typically consist of a total dose of 48-57 Gy delivered over 16-19 fractions on a Monday- through-Friday basis. For dogs with low grade (Patnaik grade I or II) MCT that are incompletely excised and then treated with adjuvant radiation therapy, long-term local control is achieved in >90%. Again, though, since surgery alone provides long-term local control in >70% of dogs, not every dog with an incompletely excised low grade MCT needs to undergo radiation therapy. Hopefully the newer grading system and prognostic panel will help us to better recognize which patients will benefit from this additional therapy.

For dogs with incompletely-excised high grade (Patnaik grade III) MCT, adjuvant radiation therapy is still a reasonable option as long as there is no gross evidence of distant metastasis. However, even after adequate local control is attained, over half will ultimately go on to develop metastasis. Perhaps the MCT prognostic panel will help identify which dogs are more likely to develop metastasis. For those with a more favorable panel, a combination of radiation therapy and chemotherapy might be best. In contrast, for those with an unfavorable panel and high risk for metastasis, perhaps the improved local control afforded by radiation therapy would not impact long-term prognosis, and chemotherapy alone would be a more reasonable option.

Radiation therapy also can be used as a primary modality for tumors that are too large or invasive for surgery to be possible. In one study, local disease was controlled for a median for 12 months. In the author’s experience, though, the duration of local disease control varies greatly
from one patient to the next, and for large, infiltrative tumors it often is substantially shorter than the reported median. Additionally, there is increased risk for degranulation reactions when gross disease is irradiated.

**Chemotherapy**

Systemic chemotherapy is indicated as a primary treatment modality for dogs with gross metastatic disease. It also is recommended as an adjuvant therapy, after adequate local/regional control has been attained, for dogs considered to be at high risk for metastasis and MCT-related mortality. However, the definition of “high risk” is still being refined. All oncologists would agree that dogs with high grade MCT should be treated with adjuvant chemotherapy. Using the new 2-tier system, when dogs with high grade MCT were treated with surgery alone, median survival was 4 months and 90% of dogs died due to their MCT. Most would also agree that dogs with unfavorable prognostic panel results also should be considered for adjuvant chemotherapy. However, questions still to answered include what to do when the prognostic variables are discordant (i.e. some indicate a favorable prognosis, and others an unfavorable one), and what to do when the tumor grade and prognostic panel disagree (i.e. a low-grade tumor with unfavorable panel, or a high-grade tumor with a favorable panel). MCT associated with the oral cavity, lips, and muzzle are associated with higher rates of regional lymph node metastasis and MCT-associated mortality. Adjuvant chemotherapy should be considered for dogs with tumors in these locations. In contrast, inguinal and perineal MCT are not inherently more aggressive. Adjuvant chemotherapy should not be recommended automatically for tumors in these locations unless warranted by other prognostic factors.

The prognostic significance of regional lymph node involvement is uncertain. Adjuvant chemotherapy should be considered for dogs with regional lymph node involvement, but if relatively few mast cells are seen on histopathology and all other prognostic variables are favorable, prognosis still might be good with local therapy alone. When 19 dogs with MCT of various grades (predominantly Patnaik grade II) were treated with surgery and radiation to attain adequate regional control, and no adjuvant chemotherapy, disease progression was identified in 6 (31%) and median progression-free survival was 1240 days (3.4 years). In another study where dogs with grade II MCT with regional lymph node metastasis were treated with adequate local therapy (surgery with or without radiation therapy) followed by CCNU, vinblastine, and prednisone chemotherapy, median disease-free interval was 2120 days (5.8 years).

Once a decision is made to pursue chemotherapy, the next decision is what drug or combination of drugs to use. Several conventional chemotherapy drugs have been evaluated as single agents and in various combinations (Table 1). Additionally, over the past few years the tyrosine kinase inhibitors (TKI) toceranib phosphate (Palladia) and masitinib (Kinaveet) have become available. These two classes of drugs work very differently. Conventional chemotherapy drugs typically interfere with some aspect of cell metabolism that is critical for progression through the cell cycle. In contrast, TKIs inhibit receptor tyrosine kinases, cell surface receptors that are important for initiating signal transduction cascades that ultimately trigger cell division. As mentioned above, up to 30% of canine MCT have mutations in the RTK Kit that result in constitutive activation. Moreover, these mutations are associated with increased risk of local tumor recurrence, increased risk of metastasis, and higher tumor proliferation indices. Kit likely represents the primary target of Palladia and Kinaveet, however tumor responses are still seen in tumors that do not have the most common genetic mutations. There are several possible explanations for this: these MCT might have other Kit mutations for which there are no
commercially available screening tests, wild-type Kit might still play an important role in MCT progression, or other RTKs targeted by Palladia and Kinavet might also play a role in MCT progression.

For dogs with tumors where Kit mutation status is known, the author currently recommends using TKI drugs as first-line therapy for Kit mutation positive tumors because they have the highest overall response rate. However, for Kit mutation negative tumors, starting with conventional chemotherapy drugs is recommended since they have higher reported overall response rates. (It is worth noting, though, that the impact of Kit mutation status on response rates with conventional drugs has not been evaluated.) For dogs with measurable disease, response to therapy can easily be assessed. However, when treating dogs in the adjuvant setting, response to treatment cannot be easily assessed unless progressive disease is identified. Some oncologists are therefore recommending that dogs in this latter group be treated sequentially with conventional drugs first followed by a TKI. Protocols using conventional drugs and TKIs concurrently are not recommended until safe dosages are established. One study demonstrated unacceptable hematologic toxicity when vinblastine and Palladia were administered concurrently.

Table 1: Efficacy of chemotherapy drugs used to treat canine mast cell tumors (measurable disease)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Number Treated</th>
<th>Complete Response (%)</th>
<th>Partial Response (%)</th>
<th>Overall Response Rate (%)</th>
<th>Median Remission Duration</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Prednisone</td>
<td>25</td>
<td>4 (assessed at 28 d)</td>
<td>16 (assessed at 28 d)</td>
<td>20</td>
<td>Not given</td>
<td>Ref 1</td>
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<tr>
<td>Vinblastine (2.0 mg/m² q1 wk)</td>
<td>25</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>77 d</td>
<td>Ref 2</td>
</tr>
<tr>
<td>Vinblastine (3.5 mg/m² q2 wk)</td>
<td>26</td>
<td>4</td>
<td>23</td>
<td>27</td>
<td>CR = 63 d PR = 28 d</td>
<td>Ref 2</td>
</tr>
<tr>
<td>CCNU (lomustine)</td>
<td>19</td>
<td>5</td>
<td>37</td>
<td>42</td>
<td>CR = 440 d PR = 77 d</td>
<td>Ref 3</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>46</td>
<td>4</td>
<td>24</td>
<td>28</td>
<td>CR = 352 d PR = 46 d</td>
<td>Ref 4</td>
</tr>
<tr>
<td>Vinblastine &amp; Prednisone</td>
<td>15</td>
<td>33</td>
<td>14</td>
<td>47</td>
<td>154 d</td>
<td>Ref 5</td>
</tr>
<tr>
<td>CCNU, Vinblastine &amp; Prednisone</td>
<td>17</td>
<td>30</td>
<td>35</td>
<td>65</td>
<td>CR = 141 d PR = 66 d</td>
<td>Ref 6</td>
</tr>
<tr>
<td>Cyclophosphamide, Vinblastine, &amp; Prednisone</td>
<td>11</td>
<td>46</td>
<td>18</td>
<td>64</td>
<td>Progression-Free Survival 74 d</td>
<td>Ref 7</td>
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<tr>
<td>Paclitaxel (Paccal Vet – water soluble micellar drug preparation)</td>
<td>168</td>
<td>1 (at 100d)</td>
<td>6 (at 100d)</td>
<td>7 (at 100 d)</td>
<td>Not given</td>
<td>Paccal Vet not commercially available. Ref 8</td>
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<tr>
<td>Toceranib Phosphate (Palladia)</td>
<td>145</td>
<td>15 (at 42 d)</td>
<td>28 (at 42 d)</td>
<td>43 (at 42 d)</td>
<td>84 d</td>
<td>ORR 60% for Kit ITD+ tumors, 31% for Kit ITD- tumors. Ref 9</td>
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<tr>
<td>Masitinib (Kinavet)</td>
<td>202</td>
<td>11 (at 168 d)</td>
<td>5 (at 168 d)</td>
<td>16 (at 168 d)</td>
<td>Time to Progression 118 days</td>
<td>Ref 10</td>
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REFERENCES


