Canine Mast Cell Tumours

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Summary
Mast cell tumours are one of the most common and clinically important skin tumours in dogs. The clinical behavior is highly variable and can include local extension, metastasis and paraneoplastic disease due to release of vasoactive substances. Depending on the tumour grade and other prognostic factors a multimodal treatment approach is necessary.

Introduction
Mast cells (MC) originate from the bone marrow, but migrate to peripheral tissues (esp. skin, GI tract, lungs) where they differentiate and mature. MC are part of the body’s immune system and involved in allergic and inflammatory reactions. MC have abundant cytoplasm containing a wide variety of vasoactive substances (e.g. histamine, heparin, etc.) stored in characteristic granules. The stored vasoactive cytokines can be released upon stimulation. Neoplastic accumulations of mast cells are called mast cell tumours (MCT). MCT are one of the most common cutaneous tumours in dogs: up to 20% of all canine cutaneous tumours

MCT are usually solitary skin tumours. Clinical presentation is extremely variable: they may be ulcerated or pruritic; some even feel like lipomas. MCT show a highly variable biologic behaviour.

Most are clinically silent skin nodules, however mast cell degranulation and release of vasoactive substances can cause local or systemic/paraneoplastic reactions including swelling, pruritus, erythema, oedema, wound healing problems, local coagulopathies or even systemic complications (gastric hyperacidity and ulceration; hypotension, anaphylactic shock, vomiting/diarrhoea, melena, systemic coagulopathies).

Diagnosis
Fine-needle aspiration cytology allows diagnosis of MCT in > 90% of cases. MC are round cells with a round to oval nucleus and abundant cytoplasm containing cytoplasmic metachromatic granules in > 90% of cases. Poorly differentiated MCT may lack granules and histology and potentially immunohistochemistry may be required for diagnosis. Cytology can give a vague direction whether the tumour is well differentiated or poorly differentiated. However, the degree of tissue invasion, which is a key criterion for accurate MCT staging cannot be assessed cytologically. Therefore, histology is always recommended for assessment of tumour grade (and surgical margins).

Biological behavior
MCT can cause problems due to local extension, metastasis and paraneoplastic disease. Several tests on histological specimens exist to predict the biological behaviour of MCT including:

1. Classical histological grading system (Patnaik et al., 1984): 3 grades (grade 1 being good)
2. New histological grading system (Kiupel et al., 2011): 2 grades (low-grade vs high-grade)
3. Mitotic Index (high mitotic index being associated with poor prognosis)
4. Ki-67 expression (high expression being associated with poor prognosis)
5. Immunohistological intracellular c-kit expression: 3 staining patterns (patterns 2 or 3 are associated with a poor prognosis).

Tests 2-5 are especially useful for grade 2 tumours according to Patnaik.

Staging
All cutaneous MCT are potentially malignant, however the likelihood of metastasis is grade dependent. MCT can metastasize lymphogenously (i.e. to the regional lymph nodes) and/or haematogenously (predominantly to the liver, spleen and bone marrow). Metastasis to the lungs is very uncommon. Useful staging tests include:

1. Palpation and aspiration cytology of regional lymph nodes
2. Ultrasound of the abdomen with a focus on liver, spleen and lymph nodes
3. Potentially aspiration cytology of liver and spleen: matter of debate
4. Chest radiographs (mainly for MCT in the cranial half of the body, to assess intrathoracic lymph node enlargement)

Not every patient requires full staging, many oncologists only recommend full staging in cases of poorly differentiated MCT, multiple MCT or MCT with confirmed lymph node metastasis.

Treatment
Therapeutic options depend on the number of MCTs present, the tumour grade, stage and location. Because of their unpredictable biological behaviour, in general, MCT should not be “monitored”
**Surgery** is the treatment of choice for localised, non-metastatic cutaneous MCT. It is essential to know before surgery if a mast cell tumour is present or not. MCT grow invasively into surrounding tissues and therefore wide resection is necessary. Marginal resection leaves tumour cells behind and tumour recurrence is likely. A repeat surgery at that stage is, if at all possible, generally more difficult and invasive. For the majority of grade I and II MCTs, a lateral margin of 2 cm and a deep margin of 1 fascial plane is sufficient to achieve complete excision. The deep margin should always include the panniculus muscle (if present), the underlying fascia or, in its absence, the superficial layer of the underlying musculature. If complete excision is achieved, most grade 1 or grade 2 tumours can be cured. All MCT larger than 3 cm and all grade 3 MCT should be excised with a lateral margin of at least 3 cm plus the deep fascial plane. Whenever surgical excision is incomplete, there are 2 options:

1) 2-3 cm re-resection around the scar including the deep fascial plane.
2) radiation therapy to “sterilize” the tumour bed.

At some locations wide surgical resection may not be feasible: e.g. at the distal limbs. Here, marginal/debulking resections followed by adjuvant radiation therapy are recommended. In case of high-grade or metastatic mast cell tumours, adjuvant chemotherapy or tyrosine kinase inhibitors are recommended. Inoperable tumours can be treated with radiation therapy or chemotherapy. In some cases such treatment can shrink the mass and make these tumours resectable. For MCT grade 1 or grade 2 with lymph node metastasis marginal resection and adjuvant radiation therapy of primary tumour bed and lymph node bed can achieve median disease-free intervals of ~ 40 months.

In case of multiple, unrelated mast cell tumours (n < 5-6) all mast cell tumours should be excised as if they were solitary MCT. If there are too many simultaneous MCT to resect, systemic therapy (chemo/TKI-inhibitors) may be more appropriate.

Radiation therapy is recommended in cases of:

- a) Incompletely resected, microscopic MCT → radiation therapy of grade 1 or grade 2 tumours is most often curative. Local control can often be achieved in grade 3 MCT, but most succumb to metastatic disease.

- b) Primary irradiation of macroscopic tumours → in most cases only palliative (partial response, stabilization).

MCT are generally moderately radiosensitive. Generally, fractionated (“curative”) radiation protocols are superior to hypofractionated (“palliative”) protocols.

Systemic chemotherapy is recommended as primary or adjuvant treatment for metastasized and/or poorly differentiated MCT (i.e. grade 3 MCT or grade 2 MCT with negative prognostic factors). The goal is tumour control or delay/prevention of metastasis. Adjuvant chemotherapy may also be useful in incompletely resected MCT if another resection or radiation therapy are not possible.

Corticosteroids are often used as part of the chemotherapy regime because of their direct inhibitory effect on MC. However, response rates of prednisolone used as a single agent are generally modest (20-40% remission) and short lived. A variety of other chemotherapeutic drugs are used including vinblastine, cyclophosphamide, lomustine, or hydroxyurea. Vinblastine and/or lomustine (CeCeNu) combined with prednisolone are the most potent agents. Response rates vary between 20-60%. Even with chemotherapy patients with grade 3 MCT generally only achieve median survival times of 7-12 months. An alternative to conventional chemotherapy are tyrosine kinase inhibitors (TKI).

**Prognosis**

Clinical aspects with prognostic relevance are mainly tumour grade and stage. Dogs with grade 1 or grade 2 MCT that were completely excised or irradiated after incomplete surgical resection achieve local control in > 90 % of cases; they are essentially “cured”. Median survival of dogs with grade 3 tumours treated with multimodality therapy is ~ 7-12 months. Treatment with TK-inhibitors may be useful and achieve long-term survival in a subset of those patients.

**Tyrosine Kinase Inhibitors**

KIT (stem cell factor receptor, CD 117) is a receptor tyrosine kinase found on the surface of all mast cells. It plays a key role in normal mast cell growth and differentiation. Mutations in the c-kit gene (which is coding for the KIT protein) have been demonstrated in 15-40% of dog with intermediate to high-grade mast cell tumours (MCTs), and these mutations result in uncontrolled signaling and tumour growth. In most cases, the c-Kit mutations consist of so called “internal tandem duplications” (ITDs) in the “juxtamembrane domain” of KIT. This domain is a key regulator of kinase function.

Mutations in c-kit have been associated with:

1. tumour grade (more commonly found in intermediate to high-grade tumours)
2. uncontrolled tumour growth
3. increased risk of local recurrence
4. decreased survival

Recently, mutations in c-Kit have been identified in some feline MCTs suggesting tyrosine kinase inhibitors may also play a role in managing this disease in cats.

**Tyrosine Kinase Inhibitors in Veterinary Medicine**

Inhibiting dysregulated (mutated) tyrosine kinases is a novel treatment option in oncology. Tyrosine
kinase inhibitors can either target specific dysregulated kinases in certain types of cancer, or they can block kinases involved in tumour angiogenesis.

In veterinary medicine 2 tyrosine kinase inhibitors (TKI) have been licensed. They are so called “small molecule kinase inhibitors” and act as intracellular competitive inhibitors at the ATP binding site of the kinase. As a result of this competition fewer ATP molecules can bind to the kinase resulting in less phosphorylation and less signal transduction. This inhibits tumour cell proliferation or angiogenesis and eventually leads to tumour cell apoptosis (programmed cell death).

**Masitinib**
Masitinib (Masivet®, AB Science) is a TKI with activity against several kinases including KIT, platelet-derived growth factor a and b (PDGFR), fibroblast growth factor receptor (FGFR3)), and a few intracellular kinases.

Masivet® has been licensed by the European Medicines Agency (EMEA) to treat non-resectable canine mast cell tumours (grade 2 or 3) with confirmed mutations of c-Kit.

In a clinical trial dogs with mast cell tumours (grade 2 or 3) treated with Masivet® did not achieve a higher rate of clinical remission than placebo-treated animals. However:

1. The time to tumour progression (TTP) was significantly longer (median: 241 days) than in placebo-treated animals (median: 83 days).
2. Also, the survival rate of dogs with inoperable mast cell tumours was significantly higher in Masivet-treated dogs than in placebo-treated dogs:
   a. at 12 months (62.1% vs 36.0%)
   b. at 24 months (39.8% vs 15.0%)

The study also revealed 2 interesting aspects:
- Masivet was more effective when used as first-line therapy (i.e. not as rescue therapy when nothing else works)
- Although it is not licensed in dogs without c-kit mutations, clinical success has been documented in some dogs without confirmed mutations suggesting additional mechanisms of actions may be involved.

**Toceranib**
Toceranib (Palladia®, Zoetis) is a TKI with activity against several kinases including KIT, vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR) and various other kinases. Toceranib has both anti-neoplastic and anti-angiogenic mechanisms of action.

In a prospective, randomized, placebo-controlled, double-blinded clinical trial of dogs with non-resectable mast cell tumours (grade 2 or grade 3) toceranib showed:
- Clinical remission in 62 of 145 dogs (43%): 21 dogs CR, 41 PR.
- 12% of toceranib treated dogs had stable disease for at least 10 weeks

On the basis of these results Palladia® was licensed by the EMEA for the treatment of canine inoperable, recurrond mast cell tumours (grade 2 or 3) independent of the c-KIT-mutation status. (However, the odds of responding to Palladia were higher in mast cell tumours with c-kit mutations).

**Adverse effects of toceranib and masitinib in dogs**
Tyrosine kinases exist in non-mutated form (wild-type) in many cells of the body and are key players in normal cell signaling. Tyrosine kinase inhibitors do not only inhibit mutated TKs, but also wild-type kinases. Therefore, TKI therapy is not selective against tumours and adverse effects can occur.

More than 75% of dogs treated long-term with toceranib had evidence of adverse effects. In one third of the cases they were severe. Adverse effects are also common with masitinib. Adverse effects are very varied including gastrointestinal irritation, cytopenias, and liver and kidney damage. Further, several other adverse effects are possible (s. table and package leaflets). In the course of treatment with TKI adverse effects were occasionally lethal. However, it is important to remember that mast cell tumours can cause paraneoplastic signs (e.g. melena, vomiting, diarrhoea) that can be indistinguishable from adverse effects caused by TKI.

In view of these adverse effects, clients have to be thoroughly informed about possible side effects of TKI and regular monitoring of CBC, BC and urine are mandatory (s. package insert for detailed protocols).

Depending on the severity of adverse effects, either a dose reduction or a drug holiday may be necessary. If severe adverse effects persist, the drug may have to be discontinued.

**References**