REVIEW

Canine cutaneous and subcutaneous mast cell tumours: a review

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Cutaneous and subcutaneous mast cell tumours are common neoplasms in the dog. While the majority can be treated with adequate local therapy alone, a subset demonstrates a biologically aggressive behaviour associated with local recurrence or metastasis. This article reviews the diagnosis and tumour staging of canine mast cell tumours alongside treatment options and the evidence supporting their use. In addition, prognostic markers are evaluated to highlight how one can recognise mast cell tumours that may behave in a biologically aggressive manner as well as the challenges of tumours that are large, infiltrative or in locations not amenable to wide surgical excision.

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INTRODUCTION

Canine mast cells (MCs) are derived from pluripotent haematopoietic stem cells of the bone marrow and operate in several biological settings throughout the body including induction of innate immune response, wound healing, hypersensitivity reactions, and antiparasitic activity (Kumar & Sharma 2010). Mast cell tumours are neoplastic proliferation of MCs and aberrations of c-Kit, including mutations, deletions, and duplications, have been implicated in both their tumorigenesis and biological behaviour (Downing *et al.* 2002). They are the most common malignant cutaneous tumour in dogs accounting for 16–21% of all skin neoplasms and are classified as either cutaneous (dermal) or subcutaneous (Bostock 1986, Rothwell *et al.* 1987).

INCIDENCE AND RISK FACTORS

The prevalence of mast cell tumour (MCT) in English primary-care veterinary practices is reported at 0.27% (Shoop *et al.* 2015). They usually occur in older dogs with the mean age of development between 8–9 years but have been described in those less than 1 year of age (Rigas *et al.* 2020). For those over 10 years of age, a 41 times greater odds of developing MCT is reported than those under 2 years (Shoop *et al.* 2015). No gender predisposition has been reported regarding the overall incidence of MCT development (London & Thamm 2020). However, male dogs are approximately 25% more likely to develop histologically high-grade MCTs compared to females, with this trend demonstrated in both intact and neutered dogs (Mochizuki *et al.* 2016). In the aforementioned study neutering was considered to have a protective effect for the development of high-grade MCT in male dogs decreasing the risk by approximately 40%, but not in female.

Among breeds, Boxers and golden retrievers are considered to have a high breed prevalence of MCT diagnosis (Table 1), while conversely, the German shepherd dog and Yorkshire terrier have reduced odds (Shoop *et al.* 2015). An association between breed and the development of histological high-grade tumours is also reported (Table 2). For example, the Shar-Pei and American Staffordshire Terrier are at high risk of developing high-grade MCT, whereas the Pug is predisposed to develop low/intermediate-grade MCTs, but rarely high-grade tumours (Mochizuki *et al.* 2016, Śmiech *et al.* 2019).

CLINICAL SIGNS

The majority of MCTs present as a solitary lesion with only 11–14% of dogs having multiple MCTs at presentation (Mullins *et al.* 2006, Murphy *et al.* 2006). The incidence rate on the trunk, perineal region, limbs and on the head and neck is reported at 28–43, 11–13, 36–40 and 11–13%, respectively (Kiupel *et al.* 2005, Berlato *et al.* 2012). Gross appearance to some degree may be associated with clinical behaviour as high-grade MCT are often rapidly growing, lack demarcation from adjacent tissue, can

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Table 1. Breed-type specific prevalence of mast cell tumour diagnosis

Increased prevalence of MCT diagnosis	Decreased prevalence of MCT diagnosis
Boxer	Jack Russel terrier
Golden retriever	Border Collie
Weimaraner	Cocker spaniel
Labrador retriever	West Highland white terrier
Pug	Yorkshire terrier
Staffordshire Bull terrier	German shepherd dog

MCT mast cell tumour

Table 2. Breed specific risk of developing histologically high-grade mast cell tumours

Risk	Breed
Low	Pug
Intermediate	Boxer, golden retriever, Labrador retriever, Staffordshire Bull terrier and Weimaraner
High	American Staffordshire Terrier, Bernese Mountain Dog, cocker spaniel, dachshund, Maltese, poodle, Rottweiler, Shar-Pei and Shih-Tzu

be ulcerated or cause irritation, present with satellite lesions, or are associated with paraneoplastic signs such as wheal formation or gastro-intestinal ulceration (Fig 1). Equally tumours greater than 3 cm in diameter, ulcerated or that are causing systemic signs of disease are significantly more likely to have nodal metastasis (Stefanello *et al.* 2015). MCT affecting the muzzle, muco-cutaneous junctions of the lip and perineum, subungal, prepuce, vulva and scrotum have been associated with an aggressive behaviour (Sfiligoli *et al.* 2005, Hillman *et al.* 2010, Elliott *et al.* 2016). In particular, regional lymph node (RLN) metastasis at the time of diagnosis is reported in approximately 50–60% of dogs with MCT on the muzzle (Fig 2) (Gieger *et al.* 2003). Regardless of macroscopic appearance, MCT should be considered as a differential diagnosis for any cutaneous or subcutaneous mass that fluctuates in size (Blackwood *et al.* 2012).

DIAGNOSTIC INVESTIGATIONS

Mature MCs have characteristic cytoplasmic granules to which their composition is influenced by the surrounding microenvironment (Noviana *et al.* 2004). As such the majority can be diagnosed from fine-needle aspiration cytology (Kiupel 2017). Cytological grading (Table 3), as a means of helping to predict tumour behaviour, has been investigated by applying the criteria of the Kiupel histological grading system as it relies on cellular morphological features, rather than on tissue architecture that the Patnaik grading system does (Camus *et al.* 2016). For cytological grading, a sensitivity of 85–88% and specificity of 95–97% is reported using histopathology as a gold standard (Camus *et al.* 2016, Hergt *et al.* 2016, Scarpa *et al.* 2016), but regrettably, only one study associates the grade with outcome (Camus *et al.* 2016). Similar sensitivity and specificity, using histopathology on the whole tumour as a gold standard, is reported for an incisional biopsy before curative intent surgery (Shaw *et al.* 2018). The

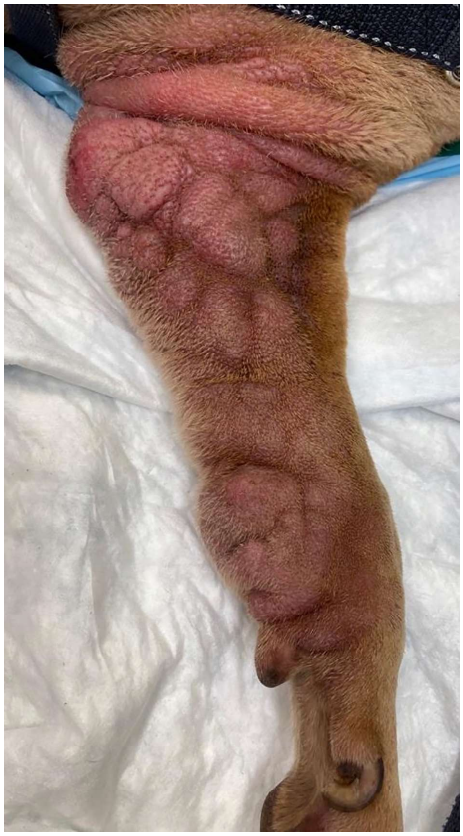


FIG 1. Poorly differentiated cutaneous mast cell tumour affecting the left forelimb of Shar Pei

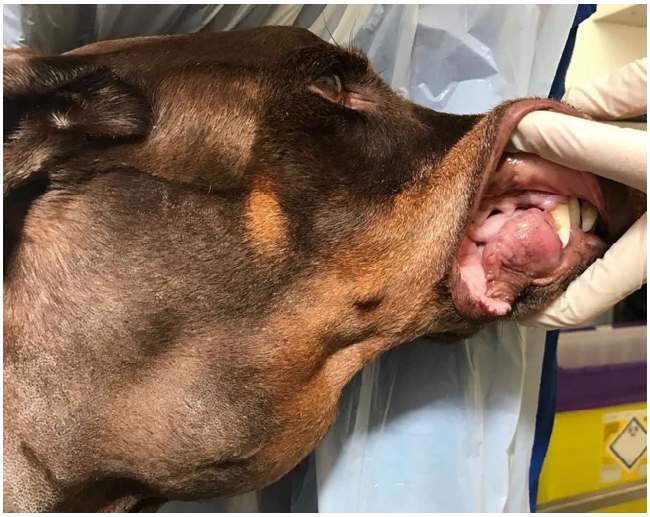


FIG 2. Mast cell tumour at the muco-cutaneous junction of the lower lip, with gross metastasis to the ipsilateral mandibular lymph node

greater advantages of cytology over incisional biopsy include less expense, being obtained without sedation, reduced tumour handling and the absence of biopsy wound healing complications. Further validation of cytological grading is required before its routine clinical application, but with the information available at this time, cytology appears reliable in identifying low-grade MCTs, while correlation with the clinical presentation and histology is necessary after the diagnosis of high-grade MCTs.

Table 3. Camus cytologic grading criteria (2016)

Criteria to assign high cytological grade
- Poor granulation
  or
- At least two of the following features:
  - Presence of mitotic figures
  - Binucleation/multinucleation
  - Nuclear pleomorphism ("noncircular")
  - Anisokaryosis (>50% difference)

Table 4. World Health Organization clinical staging system for mast cell tumours

Stage	Description
0	One tumour incompletely excised from the dermis, identified histologically, without regional lymph node involvement
I	One tumour confined to the dermis, without regional lymph node involvement
II	One tumour confined to the dermis, with regional lymph node involvement
III	Multiple dermal tumours; large, infiltrating tumours with or without regional lymph node involvement
IV	Any tumour with distant metastasis, including blood or bone marrow involvement
Substage a: Without clinical signs Substage	b: With clinical signs

STAGING

Clinical stage, following World Health Organization guidelines (Table 4), has shown to be predictive of outcome; however, this does not mean that extensive tumour staging is required in all cases (Krick *et al.* 2009). It has been proposed when an MCT is in an anatomical site amenable to wide surgical excision, and none of the aforementioned negative prognostic factors are present, that no further investigations beyond a complete blood count, serum biochemistry profile and fine-needle aspiration cytology of the RLN need be performed before excision and submission for histopathology (London & Thamm 2020). Applying this directive is often sufficient in the majority of cases; however, it relies heavily on what the clinician perceives to be the draining RLN and the accuracy of cytology in confirmation of metastatic disease. For MCTs that exhibit the negative prognostic factors recommended diagnostic tests include, in addition to the above, abdominal ultrasound plus or minus cytological assessment of liver and spleen (London & Thamm 2020). The utility of thoracic radiographs in the staging of MCTs should be considered for assessment of concurrent diseases, as they rarely demonstrate evidence of MCT metastasis (Warland *et al.* 2012). An exception to this would be assessment for sternal lymphadenopathy in cases where they would be considered an RLN, for example, MCTs located on the ventral abdominal wall or flank.

Locoregional metastasis

Although it is widely accepted that the RLN is considered to be the first site of metastasis with canine MCT, there is currently no consensus as to a superior modality on by which means it should be assessed (Blackwood *et al.* 2012). Commonly the decision for which RLN requires cytological evaluation is based on the understanding of anatomical lymphosomes in healthy dogs (Suami *et al.* 2013). In recent years, many techniques for mapping the lymphatic draining of a tumour and to individuate the sentinel lymph node have been investigated. These include using contrast-enhanced CT or ultrasound and intra-operative procedures such as gamma scintigraphy (Worley 2014, Brissot & Edery 2017, Fournier *et al.* 2021). Interestingly, in the study by Fournier *et al.* clinicians predicting the draining lymph node of an MCT based on anatomical position were correct in only 54–58% of cases. This suggests lymphoscintigraphy may be suitable for tumour located in "zones of ambiguity" where the RLN cannot be easily predicted, for example, MCTs of the head and neck, and that reliance of lysosome maps of healthy dogs may not take into consideration the potential for aberrant lymphatic drainage in the tumour microenvironment (Fournier *et al.* 2021).

Currently, the physiological size of lymph nodes among different breeds of healthy dogs remains to be defined and warrants classification. Regardless, if regional lymphadenomegaly is suspected, the index of suspicion for metastasis is high, and subsequent aspiration for cytological assessment is warranted (Marconato *et al.* 2018). Equally in RLNs considered within physiological size histologically detectable metastasis has still been documented in nearly 50% of cases (Ferrari *et al.* 2018). Therefore, cytological evaluation of RLNs irrespective of size provides valuable prognostic information as lymph node metastasis is a well-known negative prognostic indicator (Murphy *et al.* 2006, Krick *et al.* 2009). Fine-needle aspiration is largely considered minimally invasive and the cytologic-histologic concordance in the diagnosis of MCT lymph node metastasis on histopathology following a cytological diagnosis is 82% with an overall reported false-negative diagnosis of 31% (Ku *et al.* 2017).

Recently, extirpation of the RLN has been proposed as a comprehensive means of MCT staging (Ferrari *et al.* 2018). Indeed, histological assessment of the RLN would be considered superior in defining RLN metastasis status over cytological evaluation alone alongside the possible therapeutic effect of tumour burden reduction achieved from removal. Although a new classification system for the evaluation of MCT nodal metastasis has been recently developed and many laboratories adopt it; a robust correlation with the outcome is currently lacking (Weisharr *et al.* 2014). For example, the significance of this classification system, in particular, HN1/HN2 status, on prognosis remains to be fully elucidated and further studies are required to evaluate this matter (Schulman 2019).

Distant metastasis

Aspiration of the liver and spleen is currently recommended for patients with identified nodal metastasis regardless of ultrasonographic appearance (Blackwood *et al.* 2012). This is likely attributable to the fact ultrasound appearance alone is considered a poor predictor of visceral metastasis with a sensitivity and specificity of 67 and 68% in the spleen and 29 and 93% in liver, respectively (Pecceu *et al.* 2019). Regardless, cytology of a

structurally normal liver and spleen is more often negative for MCT metastasis, with a reported incidence rate of 4.1% in 386 cases of cutaneous MCT undergoing complete clinical staging (Stefanello *et al.* 2015). However, as dogs with stage IV disease are considered to have a poor prognosis with significantly shorter survival (100 vs. 291 days) than dogs without evidence of visceral mast cell infiltration this low morbidity procedure may be justified regardless of the absence of negative prognostic factors (Book *et al.* 2011). In the study by Stefanello *et al.*, 47% of dogs with early visceral metastasis did not have identifiable lymph node metastasis (Stefanello *et al.* 2015). Although an underestimation due to lack of identifying the correct sentinel lymph node is likely, visceral metastasis without detectable lymph node metastasis is reported and as such the absence of metastatic lymph node may not completely remove the need for cytological assessment of the liver and spleen (Finora *et al.* 2006, Pizzoni *et al.* 2017). Equally, it remains to be fully elucidated whether there is a predominance for visceral metastasis to occur to the liver, spleen or in equal distribution to both; however, two small retrospective studies have shown all dogs with cytological evidence of MCT infiltration of the liver also having infiltration of the spleen (Book *et al.* 2011, Stefanello *et al.* 2009, 2015).

Systemic mastocytosis

Systemic mastocytosis is rare and bone marrow sampling is not indicated for the routine staging of MCT (Moirano *et al.* 2018). Factors associated with marrow infiltration include an abnormal haemogram (for example anaemia, thrombocytopenia and circulating mast cell on blood film examination) or cases presenting for tumour regrowth, progression or new occurrence (Endicott *et al.* 2007). Ultimately prognosis is poor, with a median survival time of 43 days (Marconato *et al.* 2008) and currently, there is no satisfactory treatment for Stage IV disease (Pizzoni *et al.* 2017).

GRADING OF CANINE CUTANEOUS MAST CELL TUMOURS

Histological grade remains a cornerstone for predicting the prognosis (Patnaik *et al.* 1984, Kiupel *et al.* 2011). Using the Patnaik grading system alone it can be predicted that grade I MCTs will have an excellent long-term prognosis, whilst grade III MCTs would more likely be guarded to poor consequence to higher rates of local recurrence or less frequently the development of metastasis (Hume *et al.* 2011, Berlato *et al.* 2021). However, its limitations are more apparent in predicting the behaviour of grade II tumours, with a majority having a benign clinical behaviour but a subset being clinically more aggressive. This discrepancy may be consequence to the criterion alone or equally influenced by the subjectivity of histological features, for example, architecture, cellularity, and stromal reaction, perceived by different pathologists (Patnaik *et al.* 1984). Comparatively the Kiupel grading system criterion is more objectively defined and thus likely increases consonance between pathologists. Validation has been confirmed using the later system alone as an independent prognostic factor for predicting local recurrence, metastatic tendency, and overall survival in multiple studies (Sabattini *et al.* 2014, Donnelly *et al.* 2015, Stefanello *et al.* 2015).

In studies that have applied both grading systems (see Table 5), all grade I tumours were low-grade, and all grade III tumours were high-grade (Murphy *et al.* 2004, Sabattini *et al.* 2014, Stefanello *et al.* 2015, Horta *et al.* 2018). The Patnaik system was considered more sensitive and the Kiupel more specific in detecting dogs with aggressive disease (Stefanello *et al.* 2015). As such the question of whether using both grading systems complementary to each other to refine the prognosis for grade II tumours, subclassifying them as grade II/ low-grade or grade II/ high-grade groups, remains unanswered.

PROGNOSTIC INDICATORS

Histological grading alone is unable to predict the biological behaviour of each MCT. Therefore, on a case selection basis, molecular methods should be considered for more accurate prognostication (Sabattini *et al.* 2014). All prognostic markers can provide varying levels of risk assessment or hazard ratio but cannot be considered to have 100% positive and negative predictive values.

Mitotic count

When evaluated as an independent prognostic factor with a threshold of 5 mitoses per 10 high powered field (HPF), mitotic count (MC) has a predicted tumour-related death sensitivity of 39–55% and specificity of 86–99%. Mast cell tumours with an MC less than or equal to 5 had a median survival time (MST) of greater than 70 months, compared to MC greater than 5 reported between 2–5 months (Romansik *et al.* 2007, Vascellari *et al.* 2013, Berlato *et al.* 2015). In addition, MC has statistically been associated with

Table 5. Association of histological grade with incidence rate, incidence of metastasis, mast cell tumour (MCT)-related deaths, median survival and 1-year survival using combined Patnaik *et al.* (1984) and Kiupel *et al.* (2011) grading systems

Grade (Patnaik/ Kiupel)	Incidence (%)	Incidence of RLN metastasis at staging (%)	Incidence of distant metastasis at staging (%)	MCT related deaths (%)	Median survival time (months)	1-year survival (%)
G1/LG	13.5–24	6	2	0	Not reached	100
G2/LG	53.6–57.6	16	2	3–17	Not reached after 92 months	94
G2/HG	17.8–26	15	2	14–56	7.5–23.3 months	46
G3/HG	11.1–18	46	21	67–83.3	3.6–6.8 months	16–46
Subcutaneous	15.4	1.5	2.5	8–9	Not reached	91–95

RLN regional lymph node, G1 grade 1, G2 grade 2, G3 grade 3, LG low grade, HG high grade

metastatic rate, the higher the MC the great the risk of metastasis, but not with recurrence rate (Romansik *et al.* 2007). Although the appearance of mitotic figures in H&E preparations is considered characteristic, assessment and calculation of MC is subjective with variable inter and intraobserver agreement, for example, the targeting of mitosis "hot spots." Although the cut off value of MC greater than 5 correlating with a poorer prognosis is reproducible it has been questioned as to whether the value of 5 itself is a clinically meaningful since dichotomization of a continuous prognostic variable may in fact be considered arbitrary. This value was challenged by the suggested stratification of MC into three categories (MC 0, 1–7 and >7 per 10 HPF) where Elston *et al.* proposed that a cut off of MC greater than 7 may be a superior prognostic indicator for predicting recurrence (Elston *et al.* 2009). Conversely in the study by Berlato *et al.* that assessed the receiver operating characteristic curve in intermediate-grade MCTs demonstrated that a MC of 5 is in fact the correct threshold with the superior accuracy in predicting MCT related survival (Berlato *et al.* 2018).

Ki-67 proliferation index

Ki-67 index has been significantly associated with increased mortality, recurrence and metastasis independently from tumour grade (Abadie *et al.* 1999, Scase *et al.* 2006, Webster *et al.* 2007). It should be recognised that measurement of Ki-67 is currently not standardised between laboratories and there are differing threshold values likely consequence to the staining and counting techniques implemented. It has been proposed that Ki-67 index staining should be considered in grade II MCT with a low MC when there is discrepancy between history (rapid tumour growth or presence of paraneoplastic syndromes) or characteristic of the tumour (large size, ulceration or significant local inflammation), which might indicate aggressive disease (Berlato *et al.* 2015). Two studies showed that applying a Ki-67 threshold of 1.8% to grade II MCTs had a sensitivity of 60–79% and specificity of 83–90% in detection of dogs that will die from MCT related causes. In the univariate analysis, the risk of dogs dying with a Ki-67% greater than 1.8% was between 9.8 and 19.1 times higher than dogs with Ki-67% less than or equal to 1.8% (Maglennon *et al.* 2008, Berlato *et al.* 2015). Taking this into consideration it may be argued that dogs with lymph node metastasis (WHO stage II), distant metastasis (WHO stage IV), grade III or high-grade MCTs may not require assessment of Ki-67% given the already associated worse prognosis (Berlato *et al.* 2015).

Minichromosome maintenance protein 7

Minichromosome maintenance protein 7 (MCM7) is another proliferation marker to which its utility is comparable with Ki-67. Although MCM7 is not currently commercially available, a study suggested that mitotic count, Ki-67 index and MCM7 were statistically independent prognostic markers from one another but their combination can improve the accuracy of prognostication (Berlato *et al.* 2018).

C-KIT expression and KIT immunostaining patterns

Detection of internal tandem duplication mutation in exons 11 and 8 of the gene c-kit requires a PCR-based assay whereas expression of KIT patterns uses immunohistochemistry. Activating mutations in C-kit are generally associated with high histological grade, increased rate of local recurrence and decreased survival (Kiupel *et al.* 2004, Takeuchi *et al.* 2013). The overall incidence of c-Kit mutation in MCTs is low at 10%; however, mutations are more frequent in grades II and III MCTs *versus* grade I at an incidence of 30 and 8%, respectively (Downing *et al.* 2002, Zemke *et al.* 2002). Mutations most commonly are internal tandem duplication of exon 11 and may be a useful predictor of progression-free survival (PFS) and risk of recurrence when treated with surgery or multimodality therapy; however, this has not been validated as independent prognostic factor (Webster *et al.* 2007). In a study of dogs with internal tandem duplications, a disease-free interval (DFI) of 130 days was reached, while those without mutations had a DFI of 345 days (Takeuchi *et al.* 2013). Clinically, a positive mutation may be most informative about histologically low-grade tumours which could be biologically aggressive and further local therapy may be considered or drive treatment toward the use of a tyrosine kinase inhibitor (Thamm *et al.* 2019). Equally, dogs with patterns 2 or 3 are associated with both an increased rate of recurrence and/or decreased survival, compared to MCTs with pattern 1 when treated with surgery alone (Kiupel *et al.* 2004).

Multiple mast cell tumours

Up to 21% of dogs will present with multiple de novo MCTs, either at presentation or as subsequent events, and in most studies dogs with MCTs have no evidence of differing survival times compared to those with a single tumour (Mullins *et al.* 2006, Murphy *et al.* 2006, O'Connell & Thomson 2013). Breed predispositions have been proposed including the golden retriever and Pug (McNiel *et al.* 2006, Murphy *et al.* 2006). In the later study multiple MCTs were described in 56% of Pugs present with MCT disease, of which 94% were accredited with a low or intermediate histologic grade (McNiel *et al.* 2006). Largely, these cases would be considered patients with multiple WHO stage 1 tumours and, following surgical excision, there is currently insufficient evidence to definitively advocate the use of adjuvant therapies such as chemotherapy in decreasing the risk of further de novo MCT formation. Conversely, excluding tumour regrowth or satellite metastasis, a subpopulation of dogs have multiple cutaneous MCTs that behave in a biologically aggressive manner to be more considered in their current classification of WHO stage 3. Anecdotally discrimination between these two groups on initial presentation may initially be challenging and based on comprehensive tumour staging and histological grade adjuvant therapies may be implemented in the latter cases in attempt to maintain disease control.

Subcutaneous MCT

The majority of dogs with subcutaneous MCTs have a favourable prognosis with extended survival times from good local tumour control and low rates of local recurrence or metastasis (Table 5). Histologic grading schemes have not been developed for subcutaneous MCTs; however, decreased survival time has been linked to MC, infiltrative growth pattern and presence of multinucleation

(Thompson *et al.* 2010a, 2010b). In this study, dogs with a MC greater than 4 in 10 HPF had shorter survival times (212 days *versus* not reached), decreased time to local recurrence and metastatic rate than did those with a MC less than or equal to 4. In addition, subcutaneous MCTs with an increased Ki-67 (>23) are significantly more likely to locally reoccur and metastasise (Thompson *et al.*, 2010a, 2010b, Gill *et al.* 2020).

TREATMENT

Where possible surgery is the mainstay therapy for MCTs and curative in a high percentage of cases. However, in more challenging cases treatment recommendations vary widely. As a result of this algorithms have been proposed as a means of suggesting a more standardised treatment approach (Blackwood *et al.* 2012, London & Thamm 2020). An observed commonality of these lies in the initial consideration of whether an MCT is located in an anatomic site that amenable to wide surgical excision and as to whether local therapy is sufficient or systemic therapy is also required.

LOCOREGIONAL CONTROL

Surgical margins

Historically, it has been recommended, albeit anecdotally, to excise any MCT with a gross margin of 3 cm laterally and a one tissue plane deep margin (London & Thamm 2020). Subsequently, 23 MCTs (three grade I and 20 grade II) in 20 dogs have been evaluated for completeness of excision at 1, 2 and 3 cm margins and all tumours had complete excision at 2 cm (Simpson *et al.* 2004). This metric approach was further supported by a complete excision of 91% (21/23) grade I or II MCTs with 2 cm margins and no evidence of local recurrence in excess of 538 days (Fulcher *et al.* 2008). Albeit a good standardisation for grades I and II tumours, as grade III's were not included it was not clear to deduce this would be appropriate margin for these. Successively, a proportional approach has been described using lateral margins proportional to the maximum dimension of the MCT and one uninvolved facial plane deep margin (Pratschke *et al.* 2013a, 2013b). This study included largely grades I to II MCTs but also grade III and subcutaneous MCTs (see Table 6). Eighty-five-per-cent of tumours were completely excised and in the 15% with dirty margins only one tumour had local recurrence within a median follow time of 420 days also suggesting a largely positive outcome with this method.

Histological margins

A consensus on the definition of histological margins in veterinary oncology does not exist and the meaning of "close" or "narrow" margins can often be confusing. In human oncology, the residual tumour classification scheme, defining complete histological excision as a tumour-free margin greater than 0 mm, is considered highly prognostic in most malignant tumours and the adoption of this in veterinary medicine has recently been proposed

Table 6. Published local recurrence rates for following surgical excision

Reference	Population	Recurrence rates
Michels *et al*. (2002)	31 dogs. 20 with tumour-free margins (5xG1, 14xG2 and 1xG3), 11 incomplete tumour-free margins (4xG1, 6xG2 and 1xG3).	0 and 18% died of MCT-related disease following local recurrence at tumour-free and non-tumour-free margins respectively. Median follow up times of 43 and 21 months respectively.
Weisse *et al*. (2002)	31 dogs with completely excised G2 MCT.	11% local recurrence rate. Median follow-up time 17 months.
Murphy *et al*. (2004)	340 MCT in 280 dogs (87xG1, 199xG2 and 54xG3). Of 214 tumours 42, 19 and 39% had complete, narrow and incomplete margins, respectively.	Recurrence rate 1, 12 and 19% for G1, G2 and G3 MCT, respectively. Median follow-up time 520 days.
Simpson *et al*. (2004)	23 MCT in 21 dogs (20xG2 and 3xG1) completely excised.	No evidence of local recurrence or metastasis with a medium follow up time 351 days.
Fulcher *et al*. (2008)	16 dogs with 21 MCT (4xG1 and 19xG2). 21 completely excised and 2 incomplete.	No dogs had local recurrence with a median follow up time 379 days.
Séguin *et al*. (2008)	28 dogs (30xG2) with incompletely excised MCT.	23.3% recurred locally. Median time to local recurrence was not reached with a mean follow up time of 1713 days.
Thompson et al. (2010a,2010b)	306 dogs with subcutaneous MCT. 171 with incomplete surgical margins.	2 and 12% local recurrence rates following complete and incomplete surgical excision, respectively. Median time to local recurrence was 198 days. Median follow-up time 891 days.
Schulthesis *et al*. (2011)	100 dogs with 115 MCTs (predominantly low to intermediate grade).	No local recurrence or metastasis after excision with lateral and deep margins of 10 and 4 mm, respectively, for 27–31 months.
Pratschke *et al*. (2013a,2013b)	41 cutaneous (21xG1, 18xG2 and 2xG3; 37 LG and 4 HG) and 6 subcutaneous MCTs. 40 completely excised and 7 incomplete margins.	Local recurrence not confirmed but suspected in 2% of cases. Median follow up time of 420 days.
Donnelly *et al*. (2015)	39 high grade and 51 low-grade MCT.	High-grade tumours had 13.7 greater odd of recurrence compared to low-grade tumours regardless of their HTFM with a 35.9% recurrence rate *versus* 3.9%. 30% of the low-grade tumours had HTFM less than 3 mm, none of which recurred.

G1 grade 1, G2 grade 2, G3 grade 3, LG low grade, HG high grade, MCT mast cell tumour, HTFM histologically tumour-free margin

(Liptak 2020). Regardless, MCTs have been reported to recur within both clean and dirty margins and the percentage of MCT recurrence between studies varies (see Table 7). Examples that stress this matter include the study of 115 completely resected grades I and II MCTs in 100 dogs with histological margins of greater than or equal to 10 mm laterally and greater than or equal to 4 mm deep where no recurrence or metastasis was reported within a minimum follow-up period of 27 months (Schulthesis *et al.* 2011) compared to another paper which cites a recurrence rate 11% in 31 dogs with completely excised grade II MCTs (Weisse *et al.* 2002).

Equally, a good outcome may still be achieved in some tumours following incomplete resection, with the reported percentage of local recurrence ranging from 12% (Thompson *et al.* 2010a, 2010b) to 23.3% (Séguin *et al.* 2008). To additionally highlight the challenges associated with interpretation of MCT margins Donnelly *et al.* (2015) demonstrated that regardless of histologically tumour-free margins in high-grade Kiupel MCTs there was 36% recurrence rate compared to only 4% in low-grade tumours. For high-grade tumours, it may be suggested evaluation of tumour-free margins may be poor in the prediction of tumour regrowth. In regard to subcutaneous MCTs with complete and incomplete margins, the risk of local recurrence is low, reported at 2 and 12%, respectively (Thompson *et al.* 2010a, 2010b). When compared to their cutaneous counterparts this may suggest they are more effectively controlled by surgery alone despite incomplete excision.

Lymphadenectomy

In several studies, lymph node metastasis has been associated with a decreased survival time (Murphy *et al.* 2006, Krick *et al.* 2009, Weisharr *et al.* 2014, Pizzoni *et al.* 2017). More recently, a therapeutic value has been proposed with regional lymphadenectomy in stage II MCTs undergoing surgical removal of the primary tumour and adjuvant medical treatment (Marconato *et al.* 2018). In this study, there was a significantly reduced risk of developing local, nodal or distant relapse if the RLN was extirpated and the risk of tumour progression or tumour-related deaths was 5.5 and 3.6 times higher if the lymph node was not removed. From this, it was proposed that improved loco-regional control likely translates into a lower risk of distant spread and ultimately survival whereby undetected metastatic foci in RLN's may present a threat to disease progression. Extirpation of non-palpable or normal-sized lymph nodes may be challenging and anecdotally techniques such as ultrasound-guided intralesional injection of methylene blue (Fig 3A), intra-operative gamma probes or wire placement (Fig 3B), may improve intraoperative localisation, reduce operating time and tissue damage (Ferrari *et al.* 2020).

Radiation therapy

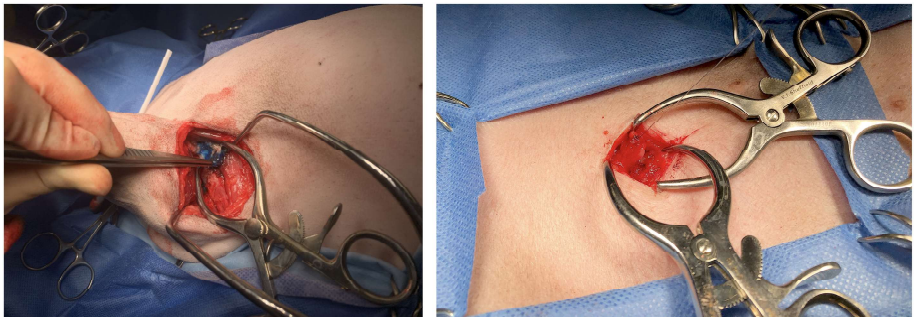
Radiation therapy is a commonly used adjuvant therapy to incompletely excised MCTs as a means of improving local control (Blackwood *et al.* 2012). Radiation prescription, including the optimal dose and fractionation regimen, is yet to be fully determined however published outcomes report a similar recurrence rate of 3–7% (Table 8). Given the low rate of recurrence described with incomplete histological margins using surgery alone the published data on the benefit of adjuvant radiotherapy remains to be fully elucidated; however, an 88% disease-free survival has been reported at 2 years for incompletely excised grade II MCTs (Al-Sarraf *et al.* 1996). One study has compared survival and local recurrence in dogs with incomplete or narrowly resected MCTs treated with no additional local therapy *versus* radiation therapy *versus* scar re-excision (Kry & Boston 2014). Recurrence rates were 38, 8 and 13%, respectively, and survival times for the adjuvant therapy groups were significantly longer than the no additional therapy group. This would support the previous argument that despite incomplete or narrow margins not all MCT recur; however, there is a likely benefit of further local therapy (Fig 4).

Radiation therapy may also be a suitable treatment option for non-surgical MCTs. For example, 35 dogs with non-resectable MCTs treated with a hypofractionated regime and prednisolone has an overall response rate of 88.5% and a 1- and 2-year PFS of 60 and 52% (Dobson *et al.* 2004). Equally, the combination of hypofractionated radiation therapy, prednisolone and toracenib phosphate has also been explored for unresectable MCTs with an overall response rate of 76% and a median progression-free interval of 316 days (Carlsten *et al.* 2012). Interestingly, this would be

Table 7. Published outcomes of dogs with incompletely excised mast cell tumour (MCT) treated with variable radiation protocols

Reference	Population	Recurrence rates
Al-Sarraf *et al.* (1996)	32 dogs with incompletely excised G2 MCT.	6% recurrence within 1 year and 12% recurrence within 2 years. Median time to recurrence of 389 days.
Frimberger *et al.* (1997)	37 dogs with incompletely excised moderately differentiated MCT.	3% recurrence within 1 year and 7% recurrence within 3 years.
Chaffin and Thrall (2002)	19 dogs with stage II MCT based on LN cytology (1xG1, 16xG2 and 2xG3).	16% local recurrence. Median disease-free survival 1240 days.
Hahn *et al.* (2004)	31 dogs incompletely excised G3 MCT.	Median remission duration 27.7 months. 1- and 2-year tumour remission rates of 65% and 26%.
Poirier *et al.* (2006)	45 dogs with incompletely excised G2 MCT.	6.7% recurrence within 7 months of treatment.
Kry and Boston (2014)	64 dogs with 70 MCT (17xG1, 48xG2 and 5xG3).	Median time for local recurrence for primary re-excision (2930 days) and RT (2158 days) were longer than the comparison group (399 days). Local recurrence recurred in 13%, 8% and 38% of cases respectively.

G1 grade 1, G2 grade 2, G3 grade 3, LG low grade, HG high grade, LN lymph node, RT radiotherapy



(A) (B)

FIG 3. (A) Surgical approach to right axillary lymph node following ultrasound guided intralesional injections of methylene blue. (B) Surgical approach following ultrasound guided wire placement into the left inguinal lymph node

Table 8. Published outcomes evaluating the efficacy of chemotherapy for "high-risk" mast cell tumour (MCT) in the post-surgical setting

Reference	Agents		Population	Response rates
Thamm *et al*. (2006)		Prednisolone Vinblastine	61 dogs (14xG2 and 47xG3) treated with surgery ±RT. 10 with residual microscopic disease and 51 with adequate local control. LN metastasis in 28 patients.	DFI 1305 days. OS not reached. 80, 70 and 65% alive at 1, 2 and 3 years, respectively. 100% "high risk" G2 alive at 3 years. OS for dogs with G3 was 1374 days.
Haynes *et al*. (2007).		Prednisolone Vinblastine	14 dogs with G3 MCT. 3 clean, 8 narrow and 3 dirty margins. 8 with nodal metastasis.	MST not reached (median follow up period of 429 days). 1- and 2-year survival probability 0.71. MST for dogs with secondary disease was 322 days and 1-year survival probability of 0.47.
Lejeune *et al*. (2013)	Prednisolone Vinblastine CCNU		21 dogs with G2 stage II MCT with adequate local control.	MST 1359 days. Median DFI 2120 days.
Horta *et al*. (2017)	"High risk"	Group A1. CCNU Chlorambucil Prednisolone	11 dogs (10xG2, 1xG3; 3xLG, 8xHG)	58.9% had disease progression. 50% died from MCT related deaths. DFI and OS of 134 and 258 days, respetivley.
		Group A2. CCNU Prednisolone	10 dogs (7xG2, 3xG3; 1xLG, 9xHG)	DFI of 686, 107 and 109 days for A1, A2 and A3, respectively. Median OS not reached, 148 days and 213 days for A1, A2 and A3 respectively.
		Group A3. Control	13 dogs (9xG2, 4xG3; 4xLG, 9xHG)	
	"Intermediate risk"	Vinblastine Chlorambucil Prednisolone	8 dogs. (8xG2; 8xLG, 0xHG)	2.5% had disease progression. 0% died from MCT related deaths. Median DFI and OS not reached.
		Chlorambucil Prednisolone	22 dogs (3xG1, 19xG2; 22xLG)	
		Control	10 dogs (10xG2; 10xLG)	
Hay and Larson (2019)	Prednisolone CCNU		15 dogs (6xG3, 9 G2/HG).	13% local recurrence, 27% de novo MCT formation and 13% developed metastatic disease. MST 904 days. 1- and 2- survival probability 0.6 and 0.4.

G1 grade 1, G2 grade 2, G3 grade 3, RT radiotherapy, DFI disease-free interval, OS overall survival, MST median survival time, CCNU lomustine, LN lymph node, LG low grade, HG high grade

considered higher than that of toracenib phosphate when used as a single agent in this setting (London *et al*. 2009).

Prophylactic radiation of RLNs is an area of radiation therapy in its infancy and whether it provides improved loco-regional control or survival benefits remain to be fully determined. Progression-free survival benefits (>2381 *versus* 197 days) have been proposed in a single study of high-grade MCTs, where only six-stage zero dogs received scar irradiation with prophylactic LN irradiation compared to 14 dogs' stage zero dogs receiving scar irradiation only and as such this area warrants further investigations (Mendez *et al*. 2019).

Tigilanol tigilate

Tigilanol tigilate (TT) is a novel diterpene ester licensed for treatment of non-resectable, non-metastatic subcutaneous MCTs located at or distal to the elbow or hock, and non-resectable, non-metastatic cutaneous MCTs measuring less than or equal to 8 cm^3 in volume. Administered as an intra-tumoral injection

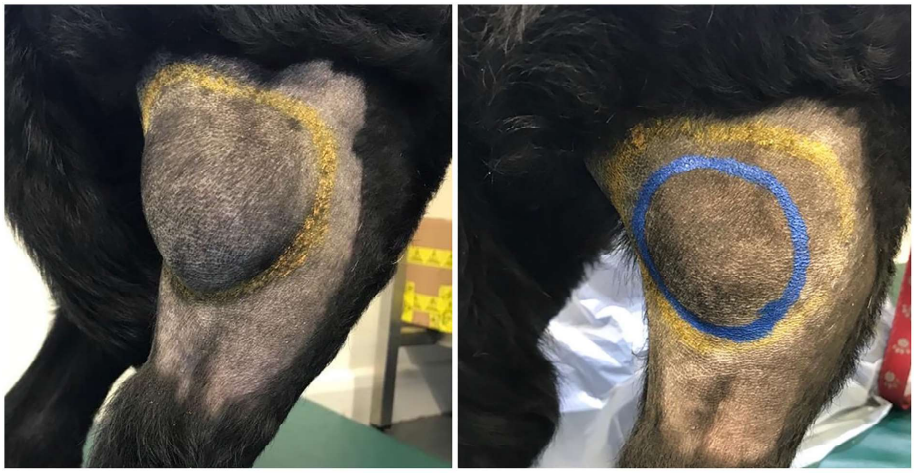


FIG 4. (A) Appearance of subcutaneous MCT on the lateral aspect of the right stifle at presentation. Contours of the tumour delineated with a yellow indelible pen. (B) Fourteen days following neoadjuvant prednisolone and lomustine administration a partial response is observed. Contours of palpable tumour burden delineated with a blue indelible pen

FIG 5. (A–C). Zero, 7 and 28 days following intra-tumoral injection of Tigilanol tigilate into a grade 2 (Patnaik) low grade (Kiupel) MCT overlying the left upper eyelid

its mechanism of action involves eliciting a rapid and localised inflammatory response, activation of the protein kinase C signalling cascade, disruption of tumour vasculature and induction of tumour death (Miller *et al.* 2019). Following separation from the surrounding tissue, the defect is allowed to heal via secondary intention with full wound closure occurring typically between 28 and 84 days (Fig 5A-C).

TT has been evaluated in a limited number of studies (Miller *et al.* 2019, De Ridder *et al.* 2020). In the study by De Ridder of 123 canine MCTs, a complete response rate of 75% was reported with a single intra-tumoral injection with no evidence of local recurrence in 93% of dogs by 84 days. When including dogs that received that second administration of TT an 87.2% complete response rate was achieved (De Ridder *et al.* 2020). The most commonly reported adverse events include wound formation at treatment site and injection site bruising, erythema and oedema, both essential indicators of treatment (Miller *et al.* 2019). TT poses as an attractive alternative treatment for MCTs in locations that are not amenable to wide surgical excision or in patients who may have comorbidities that pose a high risk for general anaesthesia; however, limitations include the lack of histopathological tumour grading, reported margins and documented long-term responses. At this time, it is likely TT may be most suited to stage 0 MCTs that display traits of being more likely to behave in biologically low-grade manner.

SYSTEMIC THERAPIES

Adjuvant chemotherapy

Systemic treatment with chemotherapy is conventionally used for high-grade tumours or intermediate-grade tumours with features suggesting a high risk of malignancy. As an adjuvant therapy, the goals of its use are to delay or prevent metastatic disease, provide further loco-regional control and reduce the risk of de novo MCT formation. (Blackwood *et al.* 2012, O'Connell & Thomson 2013). A plethora of published chemotherapy protocols exist (Tables 9 and 10) and the reader is reminded the choice of medical therapy should take into account the individual toxicities each drug may pose to the patient (Blackwood *et al.* 2012).

Table 9. Evaluated responses of mixed population studies including macroscopic and microscopic mast cell tumour (MCT) disease

Reference	Agents	Population	Response rates
Thamm *et al*. (1999)	Prednisolone Vinblastine	18 dogs with gross disease (18xG2, 23xG33).	57% 1- and 2-year disease-free rate
		23 dogs treated in adjuvant setting.	33% CR, 13% PR, 47% ORR. Median response duration 154 days (range 24 to >645 days).
Camps-Palau *et al*. (2007)	Prednisolone Vinblastine Cyclophosphamide	11 dogs with gross disease (7xG3, 4xG2). 10 with RLN MET (1 unknown)	45% CR, 18% PR, 18% SD, 18% PD. Medium progression-free survival 74 days. MST 145 days.
		24 dogs (22xG2 and 2xG3). 9 without adequate local control following surgery. 12 with RLN METs and 1 liver/spleen METs	Medium PFS 865 days. MST >2092 days.
Cooper *et al*. (2009)	Vinblastine CCNU	37 gross disease, i.e. measurable disease at primary tumour or a MET site (12xG2, 17xG3 and 8 no-grade available).	24% CR, 32% PR, 12% SD, 4% PD. 57% ORR for median duration of 52 weeks. Median PFS 30 wks and OST of 35 weeks.
		20 microscopic disease i.e. incompletely or completely resected G2 tumours with MI >5 or regional LN MET (8xG2, 11xG3 and 1 no-grade available).	Median PFST and OST 35 and 48 weeks respectively. G3 tumours had a 39-week median OST where all dogs with microscopic G2 tumours were still alive.
Rassnick *et al*. (2010a, 2010b)	Vinblastine CCNU Prednisolone	17 non resectable (6xG2, 8xG3, 1xnasal, 1xjejunal and 1xnot specified). 12 with RLN MET and 6 with distant MET.	29% CR (median 141 days), 35% PR (median 66 days) and 65% ORR.
		35 adjuvant treatment (17xG2, 15xG3 and 3xgastrointestinal MCT). 25 dogs with RLN MET. 7 dogs had definitive RT. 25 categorised as ALC and 10 without ALC.	Median PFS 489 days. Dogs with G3 had a shorter PFS compared with dogs with MET G2 (190 days *versus* 954 days).
Miller *et al*. 2014	Variable (predominantly Vinblastine and prednisolone or masitinib).	94 dogs (45xG2 and 44xG3). 26 with gross disease. 68 adjuvant treatment (30 wide, 16 narrow and 22 incomplete margins respectively). 51 RLN MET and 16 distant MET.	Surgically excised G2 and no known MET treated with vinblastine and prednisolone showed a significantly longer survival (MST: 1946 days) than those treated with masitinib (MST: 369 days). Improved survival in dogs with G3 when using masitinib (MST: 278 days) when compared with vinblastine and prednisolone (91 days) when treating gross disease.
Olsen *et al*. 2015	Vinblastine Toceranib Prednisolone	Neoadjuvant. 16 dogs (14xG2 and 2xG3; 13xLG; 1xHG). MET in 50%.	38 CR, 88% ORR. MST not reached (median follow up time 287 days). 25% died from MCT related causes.
		Adjuvant therapy following surgical resection. 11 dogs (7xG2 and 6xG3; 7xHG). 27% had MET at presentation. Clean surgical margins achieved in 82%.	Disease progression occurred in 36%. MST for complete and incomplete surgical margins were 893 days and 181 days respectively. PFI not reached for complete surgical margins and incomplete had a PFI of 154 days.
		Gross metastatic disease. 13 dogs (11xG2 and 2xG3; 6xLG 5xHG).	23% CR, 69% PR, 92% ORR. MST 218 days. Progression-free interval 45 days.

G1 grade 1, G2 grade 2, G3 grade 3, RT radiotherapy, CR complete response, PR partial response, ORR overall response rate, RLN MET regional lymph node metastasis, SD stable disease, PD progressive disease, MST median survival time, PFI progression-free interval, PFS progression-free survival, PFST progression-free survival time, CCNU lomustine, OST overall survival time, LG low grade, HG high grade

The survival advantage of adjuvant chemotherapy for specific tumour grades, stage I disease or in the face of HN0–HN2 metastasis have been challenged. In one study by Moore *et al.* the use of single-agent vinblastine, combination chemotherapy (vinblastine and lomustine) or toceranib protocols did not affect median survival time in dogs with high-grade Kiupel, stage 1 MCTs compared to dogs receiving no adjuvant treatment following surgery with a MST of 1128 days for dogs treated with chemotherapy *versus* 1179 days in dog that did not receive chemotherapy. Local recurrence was reported in 18.4% and RLN metastasis in 12.2%, again emphasising that local control of high-grade MCTs is important in improving survival (Moore *et al.* 2020). In addition, no survival benefit was suggested with the use of adjuvant medical therapy, chemotherapy and/or tyrosine kinase inhibitors, in dogs with low grade Kiupel MCTs with HN2 nodal metastasis following surgical excision (Marconato *et al.* 2020).

Adjuvant treatment with residual disease

Following incomplete excision, when revisional surgery or radiation therapy is not possible, adjuvant chemotherapy may be considered as an alternative treatment modality (Table 10). In two studies, treating microscopic residual disease for incompletely

Table 10. Published outcomes evaluating the efficacy of adjuvant chemotherapy for incompletely excised mast cell tumour (MCT)

Reference	Agent	Population	Response rates
Davies *et al*. (2004).	Prednisolone Vinblastine	27 dogs (24xG2; 3xG3). 19 incomplete excision, 7 marginal excision (2–5 mm narrowest margin).	10% overall local recurrence rate. Minimum of 12-month follow up period for dogs that completed the protocol. 25% developed distant cutaneous MCT.
Hosoya *et al*. (2009)	Prednisolone Lomustine	12 dogs with incompletely excised G2 MCT. 2 with RLN metastasis.	No dogs developed local recurrence or regional/ distant metastasis. Medium follow-up time 620 days. 8% developed distant cutaneous MCT.

G1 grade 1, G2 grade 2, G3 grade 3, LG low grade, HG high grade, RLN regional lymph node

excised grades II and III MCTs, local recurrence was observed in 0–10% of dogs (Davies *et al.* 2004, Hosoya *et al.* 2009). Conversely, it can be argued for incompletely excised intermediate-grade tumours the recurrence rate following surgery alone is low and that active surveillance alone may be appropriate (Blackwood *et al.* 2012). The latter however would not be considered appropriate in the event of macroscopic residual disease, tumour regrowth or subsequent development of regional or distant metastasis.

Neoadjuvant chemotherapy

For tumours in challenging locations, such as distal limb or face, where wide surgical excision or radiotherapy may not initially be possible the use of systemic treatments have been described to down-stage disease before definitive treatment (Blackwood *et al.* 2012). In a study by Stanclift of 49 dogs, the overall response rate of neoadjuvant prednisolone was 70% with a median sum maximal diameter reduction of 45% (Stanclift & Gilson 2008). The use of chemotherapy, tyrosine kinase inhibitors and radiotherapy before surgery has proven rewarding on a case selection basis, although this remains largely anecdotal (Fig. 4A,B). In a study of 16 dogs with grades II and III MCTs, 88% had a measurable response to chemotherapeutic downstaging with prednisolone, vinblastine and toceranib phosphate (Olsen *et al.* 2015). A theoretical advantage of an observed response to chemotherapy in this setting would be the continued use, if indicated, in the post-operative setting. However, as the majority of protocols used to treat MCTs in a gross disease setting also use prednisolone, it is inherently difficult to determine which agent, or if all, are ultimately contributing to the initial response observed. Equally, it remains to explore what impact neoadjuvant chemotherapy has on the histological assessment of tumour-free margins or features such as mitotic count which ultimately may influence its assigned grade.

Primary chemotherapy

Dogs may present with disease unsuitable for resection owing to size, location, presence of dissemination or concurrent morbidities preventing general anaesthesia. When treating measurable MCTs the durability of response poses a significant challenge and ultimately tends to be short lived (Table 11), stressing the importance for adequate local control (Blackwood *et al.* 2012). Therefore, the use of chemotherapy in this setting is principally palliative. Although it is suggested multiagent protocols may confer a higher response rate than single-agent therapies their increased risk of toxicity and a proven survival advantage has not been verified (Camps-Palau *et al.* 2007).

Tyrosine kinase inhibitors (TKIs)

Activating mutations of the c-kit proto-oncogene are associated with the pathogenesis and aggressiveness of MCT, resulting in phosphorylation of the KIT receptor tyrosine kinase (Zemke *et al.* 2002, Letard *et al.* 2008, Webster *et al.* 2006). Both masitinib mesylate and toceranib phosphate have been licensed for treatment of non-resectable intermediate and high-grade MCTs by targeting KIT and PDGFR, with toceranib also targeting VEGFR2. For dogs that possess the c-kit mutation that are treated with toceranib phosphate an objective response is twice as likely, 60 *versus* 31.3%, than those without. Equally, tumour grade or presence of lymph node metastasis has not been associated with objective response (London *et al.* 2009); however, the effect of c-kit mutation on PFI remains to be fully evaluated (Thamm *et al.* 2020). Given that KIT mutation status does not guarantee or conversely rule out possible response to toceranib one may argue the ultimate influence that KIT testing may have as a driving factor toward treatment decision. For dogs treated with masitinib, the association of c-kit mutation on response to treatment remains to be explored; however, a positive association has been observed between mutation status and PFI (Hahn *et al.* 2008, 2010). As with conventional chemotherapy, the durability of response in the macroscopic disease setting continues to pose a significant challenge and for the majority of patients tends to be short lived (Tables 9 and 11). In a study by Miller *et al.* which reviewed treatment and response of grade III MCTs with the gross disease a statistically significant improvement in MST was seen masitinib (278 days in 7 dogs) compared to a vinblastine and prednisolone protocol (91 days in 3 dogs) (Miller *et al.* 2014). Conversely when comparing the two treatments on excised grade II MCTs, with high Ki67% and no evidence of metastatic disease, the significantly improved MST was observed with vinblastine and prednisolone (MST 1946 days in 14 dogs) compared to masitinib (369 days in 6 dogs). Therefore, chemotherapy may be considered as the primary adjuvant treatment in a microscopic disease setting when the aim is curative intent where as TKI's are likely superior when used as palliative treatment in dogs with the macroscopic disease.

REPEAT TUMOUR STAGING

Following curative intent treatment routine follow-up is recommended as a means of active surveillance. For clinical stage 0 and I MCTs of low to intermediate grade this includes repeated

Table 11. Evaluated response rates of measurable mast cell tumour (MCT) to various agents

Reference	Agent	Population	Response rates
McCaw *et al*. (1994)	Prednisolone	25 dogs (all tumours with tissue available for grading were G2 or G3). 13 dogs had tumour recurrence following surgery and 5 grossly enlarged RLN MET.	4%CR, 16% PR, 20% ORR, Median response duration not reported.
Gerritsen *et al*. (1998)	Cyclophosphamide Vincristine Hydroxyurea Prednisolone	17 dogs (12xG2 and 5xG3). 9 stage II and 7 stage IV.	23% CR, 35% PR, 59% ORR with median response duration of 53 days.
Rassnick *et al*. (1999)	Lomustine	23 dogs (1xG1, 10xG2 and 8xG3).	5% CR, 37% PR, 32% SD, 42% ORR. Median duration of response for PR and SD was 77 and 78 days respectively.
Hahn *et al*. (2008)	Masitinib	161 dogs (138xG2 and 23xG3).	26% CR 29% PR 55% ORR with median response duration not reached.
Rassnick *et al*. (2008)	Vinblastine (2.0 mg/m^2 weekly for 4 treatments then biweekly for 4 treatments)	25 dogs (13xG2, 11xG3 and 1 unspecified). 15 dogs had RLN MET.	12% PR for median response duration of 77 days (range 48–229 days).
	Vinblastine (3.5 mg/m^2 biweekly for 5 treatments)	26 dogs (11xG2, 15xG3). 16 dogs had RLN MET.	4% CR, 23% PR for a median of 28 days (range 28–78 days), 27% ORR.
Vickery *et al*. (2008)	Prednisolone Vinblastine (dose escalating)	24 dogs (12xG2, 3xG3, 3 unspecified and 6 diagnosed by cytology). 17 RLN MET and 1 MET to liver.	VBL+Pred: 23% CR, 30.8% ORR, PFI 49 days. VBL+Pred + RT: 25% CR, 75% ORR, PFI 57 days.
London *et al*. (2009)	TOC	145 dogs (2xG1, 110xG2 and 38xG3).	14 CR% 28% PR 43% ORR with median duration of response of 12 weeks.
Taylor *et al*. (2009)	Chlorambucil Prednisolone	21 dogs (13xG2, 6xG3 and 2 diagnosed by cytology alone). 6 RLN MET, none had distant MET.	14% CR, 24% PR, 42% SD, 19% PD. 38% ORR with a median PFI of 533 days for responders.
McCaw *et al*. (1997)	Vincristine	27 dogs (16xG2 and 11xG3).	0% CR 7% PR 19% SD 7% ORR. Median response duration not reported.
Rassnick *et al*. (2010a, 2010b)	Hydroxyuea	46 dogs (22xG2, 21xG3 and 3 diagnosed by cytology alone). MET in 32 dogs.	4% CR, 24% PR, 28% ORR. Median response duration of 46 days for partial responders.
Burton *et al*. 2015	CCNU Toceranib	41 dogs. 26% local LN MET, 5% distant MET.	10% CR, 36%, 46% ORR with median PFS 52 days.
Grant *et al*. (2016)	Masitinib	39 dogs (19xG2, 10xG3, 3x subcutaneous, 7x cytological diagnosis). 54% with MET.	38% CR 44% PR 82% ORR with median response duration not reached.
Bavcar *et al*. (2017)	CCNU Toceranib	10 dogs. Primary or recurrent, non-resectable and/or metastatic Patnaik grade II/II MCT.	30% CR, 20% PR, 50% ORR with median PFS of 86 days.

G1 grade 1, G2 grade 2, G3 grade 3, CR complete response, PR partial response, ORR overall response rate, RLN MET regional lymph node metastasis, SD stable disease, PD progressive disease, PFS progression-free survival, OST overall survival time, RT radiotherapy, LG low grade, HG high grade, TOC toceranib phosphate, CCNU lomustine

physical examination and loco-regional assessment of lymph nodes initially 1, 3, 6, 9, 12, 15, 18 months post-treatment and every 6 months thereafter. For biologically aggressive MCT, the addition of abdominal ultrasound is warranted at time of reassessment (London & Thamm 2020). Although the importance of restaging is clear for assessing response to treatment the guidelines regarding timing of restaging and duration for which tumours in clinical remission are assessed for are largely anecdotal and may benefit from future refining.

CONCLUSION

The majority of MCTs are cured with appropriate local treatment and for this surgery remains the mainstay modality. The remaining subset may pose either challenging to treat or to predict whether the tumour may behave in a clinically aggressive manner. With this being said, tumour grade must not considered as the only prognostic factor and should be interpreted in combination with clinical presentation, WHO stage, additional prognostic markers and completeness of surgical margins. Equally, the presence of multiple MCTs is not always associated with a poorer prognosis and not all incompletely resected MCTs may require adjuvant treatment.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people of organisations that could inappropriately influence or bias the contents of this review.

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