

# Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment

Monika M. Welle\*, Carla Rohrer Bley†, Judith Howard‡ and Silvia Rüfenacht§

\*Institute of Animal Pathology, Vetsuisse Faculty and University of Berne, Berne, Switzerland

†Section of Diagnostic Imaging and Radiation Oncology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland

‡Diagnostic Laboratory, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Berne, Berne, Switzerland

§Assistant Professor, Dipl. ECVI, Dermatology Unit, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Berne, Berne, Switzerland

Correspondence: Monika M. Welle, Postfach 8466, Länggassstr. 122, CH-3001 Berne, Switzerland. E-mail: monika.welle@itpa.unibe.ch

## Abstract

**Mast cells (MCs) are well known for their neoplastic transformation in solitary and multiple cutaneous mast cell tumours (MCTs), as well as visceral and systemic mastocytosis. Dogs have a unique risk of developing cutaneous MCTs, and they account for 7% to 21% of all canine skin tumours. The aetiology of canine MCTs is unknown but is probably multifactorial. This article reviews up-to-date knowledge on the pathogenesis, the clinical presentation, the clinical prognostic factors, the diagnostic workup including clinical staging, cytological findings, histological findings and the various grading systems which have been evaluated based on morphology, the assessment of proliferation markers and other factors such as vessel density. Furthermore, detailed information about current treatment protocols for canine cutaneous MCTs is provided.**

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## Introduction

Mast cells (MCs) are found in most organs and tissues of the body and are present in highest numbers in locations that interface with the environment, such as the skin, the lung and the gastrointestinal tract.<sup>1</sup> It is estimated that if all MCs in the human body were assembled in one organ, their mass would equal that of the spleen.<sup>2</sup>

Mast cells are derived from CD34 progenitor cells in the bone marrow.<sup>3</sup> Although most cells descending from progenitors in the bone marrow differentiate into mature cells before leaving the bone marrow, MCs leave as morphologically unidentifiable progenitors and differentiate into phenotypically identifiable MCs only after they infiltrate connective or mucosal tissue.<sup>4–6</sup> In addition, fully granulated

MCs in well-differentiated mast cell tumours (MCTs) can undergo mitosis, indicating that fully differentiated MCs have the potential to proliferate.<sup>7</sup>

In rodents, formalin-resistant connective tissue type MCs (CTMC), and formalin-sensitive mucosal type MCs (MMC) have been identified.<sup>8,9</sup> Canine MC heterogeneity based on formalin sensitivity has been identified in the skin,<sup>10,11</sup> trachea, lung, intestine and in MCTs.<sup>11,12</sup> However, the classification used in rodents is not found to be appropriate for subtyping canine MCs.<sup>13,14</sup> Instead, three subtypes exist in dogs based on their content of the proteases, chymase and tryptase, which are present in both normal and neoplastic MCs.<sup>11,15</sup>

Mast cells can be recognized in tissues by their cytoplasmic granules when appropriately fixed and stained with cationic dyes that bind granule proteoglycans, resulting in metachromasia.<sup>16</sup> These granules store mediators of inflammation, including histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid, conferring to MCs an important role in immunological, inflammatory and immediate-type allergic reactions.<sup>17–21</sup> Extracellular release of MC granule contents can be precipitated by physical or chemical means, such as heat, trauma and toxins, or by immune mechanisms, such as antigen-specific IgE binding to IgE receptors on the MC cell surface.<sup>16,22–28</sup> In addition, MCs are well known for their neoplastic transformation in solitary and multiple cutaneous MCTs, as well as in visceral or systemic mastocytosis.<sup>29,30</sup> The biological behaviour of MCTs is highly variable, and improvements in understanding of the natural history and prognostic indicators as well as the indications for multimodal therapy will further result in better outcomes in canine patients with MCTs.

## Pathogenesis of MCTs

The aetiology of MCTs is unknown but, as with most neoplasms is probably multifactorial, and the well-documented breed predispositions likely indicate an underlying genetic component. Recent work has implicated the stem cell factor receptor, KIT, in the aetiology of canine MCTs.<sup>31</sup> KIT is a surface growth factor receptor, normally expressed on MCs and encoded by the proto-oncogene *c-kit*.<sup>32</sup> KIT consists of an extracellular ligand-binding domain, a transmembrane region and a cytoplasmic tail with ligand-dependent tyrosine kinase activity. Activated KIT binds and phosphorylates intracellular substrate proteins, initiating a signalling cascade that culminates in a wide array of biologic activities including proliferation, migration, maturation, and survival of haematopoietic stem cells, MCs, melanocytes, and germ cell lineages.<sup>32–38</sup> The ligand for KIT is stem cell factor, also known as steel

factor, KIT ligand, or mast cell growth factor.<sup>39–42</sup> KIT expression has been demonstrated immunohistochemically in both normal and neoplastic canine MCs, with higher expression in poorly differentiated MCTs.<sup>31,43–45</sup> In addition, dissimilar cytoplasmic and cell membrane expression of KIT has been found between normal and neoplastic canine MCs, and aberrant cytoplasmic KIT expression due to *c-kit* mutations has been associated with poor clinical outcome.<sup>45–47</sup> Several authors have reported a variety of *c-kit* mutations in canine MCTs, particularly in exon 11, including different point mutations and tandem duplications in the juxtamembrane coding region.<sup>35,44,45,48–54</sup> However, mutations have not been described in other regions, in particular in the *c-kit* kinase domain which is the site of the most frequent mutation in human MCTs.<sup>55–57</sup> Moreover, internal tandem duplications reported in canine MCTs have not been reported in the *c-kit* gene of human beings. Various studies have shown that about 15% to 40% of all canine MCTs are affected by *c-kit* mutations.<sup>35,44,45,48–54</sup> In addition, a significant association between mutations and tumour grade was demonstrated in two large studies in which none of 24 and one of 12 well-differentiated, eight of 58 and 42 of 119 intermediate grade, and four of six and nine of 26 high grade tumours, respectively, were affected.<sup>48,51</sup>

Mutations of *c-kit*, characterized by internal tandem duplications, produce a constitutively activated KIT protein in the absence of ligands<sup>35,49,58</sup> that leads to extracellular signal-regulated kinase phosphorylation.<sup>58</sup> Little is known about the consequences of these mutations. However, *c-kit* mutations and aberrant KIT protein localization are associated with increased expression of Ki67 and argyrophilic nucleolar organizing regions (AgNORs), both of which are markers of increased cellular proliferation.<sup>47</sup> Moreover, *in vitro* testing of drugs targeting the KIT tyrosine kinase receptor demonstrated inhibition of proliferation of canine MCT cell lines and primary neoplastic MCs, associated with cell-cycle arrest and apoptosis.<sup>59</sup> Despite these findings, no genetic defects in *c-kit* are identified in more than 60% of canine MCTs. This suggests that, mutations in this gene are associated with the development or progression of some MCTs, but mutations in other genes are likely to be involved in the initiation and progression of most canine MCTs.

In addition to genetic mutations, some authors have suggested that chronic cutaneous inflammation may play a role in MCT development.<sup>60</sup> However, only rare cases of MCTs associated with chronic dermatitis, scar formation or the application of skin irritants have been documented.<sup>61,62</sup> Other studies have suggested a possible viral cause and MCTs have been transplanted to very young or immunocompromised laboratory dogs through tumour cells and cell extracts but unequivocal evidence of a viral aetiology is lacking.<sup>63–65</sup>

## History and clinical presentation

The vast majority of canine MCT occur in the dermis and subcutaneous tissue. In addition, they are occasionally reported in extracutaneous sites, including the conjunctiva, salivary gland, nasopharynx, larynx, oral cavity, gastrointestinal tract, urethra and spine.<sup>66</sup> Visceral MCTs (disseminated or

systemic mastocytosis) are almost always preceded by an undifferentiated primary cutaneous tumour, and primary MC leukaemia is extremely rare.<sup>66,67</sup>

## Incidence

Dogs have a unique risk of developing cutaneous MCTs<sup>68</sup> which account for 7% to 21% of all canine skin tumours.<sup>69–76</sup> Furthermore, MCTs are reported to be the most common canine skin tumour submitted for histology and diagnosed in veterinary teaching hospital populations<sup>68–70,72,77</sup> with an incidence of 129 in 100 000 dogs per year in one UK study of insured dogs.<sup>78</sup>

## Breed predisposition

Breeds found to be predisposed to develop MCTs include boxers, Boston terriers, bull terriers, bullmastiffs, cocker spaniels, Staffordshire terriers, fox terriers, English bulldogs, dachshunds, Labrador retrievers, golden retrievers, beagles, pugs, schnauzers, shar-peis, Rhodesian ridgebacks, Weimaraners and Australian cattle dogs.<sup>68,70,72,79–82</sup> In one study, boxers and Boston terriers represented more than 50% of dogs with MCTs and the relative risk of developing MCTs was found to be 16.7 for boxers and 8.0 for Boston terriers.<sup>69</sup> The increased incidence in these two breeds may be related to their common bulldog ancestry.<sup>80</sup> Site predilection has also been reported for various breeds: hind limbs and multiple lesions in boxers and pugs, hind limbs in Boston terriers and American Staffordshire terriers, the tail in Rhodesian ridgebacks, multiple lesions in Weimaraners and golden retrievers, and the head and hind limbs in English setters.<sup>79,83</sup>

## Gender

Most studies report no gender predilection<sup>66,69,80–82,84</sup> or association between gender and survival,<sup>81</sup> with the exception of one European study in which female dogs were found to have a more favourable prognosis with chemotherapy than their male counterparts.<sup>85</sup> It is therefore unclear whether sex hormones may have an impact on tumour behaviour.<sup>66</sup>

## Age

The mean age of dogs at presentation is between 7.5 and 9 years,<sup>60,66,68,69,73,81,82,84,86–89</sup> although MCTs are occasionally found in dogs as young as 4 to 6 months of age.<sup>73,90,91</sup>

## Tumour site and gross appearance

Dogs with cutaneous MCTs are most frequently presented for the evaluation of a cutaneous mass, and only rarely for clinical signs related to the release of mediators from MC granules.<sup>67,68,74</sup> Cutaneous MCTs are most common on the trunk (50–60%), followed by the extremities (25–40%), and the head and neck (10%).<sup>68–70,92,93</sup> The scrotum, perineum, back and tail are less commonly affected.<sup>79</sup> In general, a greater number of tumours affect the posterior part of the body (hind limbs, perineum and prepuce).<sup>67,71</sup> The gross appearance of MCTs varies widely and can mimic other cutaneous tumours and non-neoplastic conditions (Fig. 1). MCTs must therefore be considered in the differential diagnosis of any skin nodule.<sup>86</sup> The gross appearance of MCTs correlates to some extent with histological grade.<sup>92</sup> Low-grade, well-differentiated MCTs



**Figure 1.** Six-year-old Staffordshire bullterrier with a mast cell tumour on the ear margin. An alopecic firm and exophytic nodule of 8 mm in diameter is visible.

tend to present as a solitary, rubbery, slowly growing nodule with 1 to 4 cm in diameter, which are often present for over 6 months prior to diagnosis.<sup>66,72</sup> These may be alopecic but are typically nonulcerated. Another form of MCTs are subcutaneous tumours that may be soft and fleshy on palpation and are often grossly misdiagnosed as lipomas.<sup>66</sup> Undifferentiated MCTs tend to grow rapidly, ulcerate, cause considerable irritation and may give rise to small satellite nodules in the surrounding tissues.<sup>66,72</sup> Most MCTs are not pigmented, but occasionally erythematous and hyperpigmented nodules are found.<sup>68</sup> The surrounding tissue may be inflamed and oedematous<sup>66</sup> and can present urticarial swelling or diffuse areas of oedema and inflammation resembling cellulitis.<sup>86</sup> Lesions on the distal extremities, lips and in the inguinal area may present as poorly defined areas of swelling or mimic acral lick dermatitis.<sup>29,61,94</sup> In addition, diffuse MCTs can produce gross distension and deformation of the hind limbs in shar-peis.<sup>95,96</sup>

Palpation of MCTs occasionally causes degranulation with release of histamine and other vasoactive substances that results in local vasodilatation, oedema and erythema, also known as Darier's sign.<sup>66,68,86</sup> Likewise, manipulation of visceral MCTs can cause vomiting and abdominal pain.<sup>60</sup>

### Multiple MCTs

In most dogs, MCT presents as a single cutaneous tumour, but between 5% and 25% of dogs are affected with multiple skin tumours that may occur synchronously or sequentially.<sup>29,61,66,68,71,73,83,86,89,97,98</sup> Breeds reported to be more frequently affected by multiple tumours include boxers, pugs, Weimaraners, golden retrievers and shar-peis.<sup>79,83</sup> A case of multiple pruritic MCTs that spontaneously regressed was also reported in a 3-week-old Jack Russell terrier.<sup>90</sup> However, this may have been a hyperplastic MC proliferation<sup>29,66,68</sup> or possibly a condition similar to urticaria pigmentosa in humans.<sup>86</sup>

### Paraneoplastic syndromes and complications of granule release

Complications related to the release of the bioactive substances in MC granules (histamine, heparin, eosinophilic chemotactic factor and proteolytic enzymes) occur in up to one half of dogs with MCTs.<sup>74,97</sup> Delayed wound healing

after surgical excision is a common problem attributed to proteolytic enzymes and vasoactive amines released by the tumour.<sup>66,67,87</sup> Studies in mice suggest that released histamine binds to H<sub>1</sub> and H<sub>2</sub> receptors on macrophages causing the release of fibroblastic suppressor factor that decreases normal fibroplasia and delays wound healing.<sup>66</sup> Furthermore, histamine and human mast cell leukaemia lysates have been shown to reduce keratinocyte proliferation and thus inhibit wound epithelialization.<sup>66</sup> Local haemorrhage is frequently encountered during surgical excision, fine-needle aspiration and excessive manipulation of MCTs<sup>64</sup> and is likely due to coagulation defects caused by released heparin.<sup>64,66,67,99,100</sup> Indeed, prolonged coagulation time of blood taken from or around MCTs is frequently observed,<sup>66</sup> although prolongation of coagulation times from venous blood samples is observed in a small minority of patients.<sup>99</sup> Since haemorrhage can be a serious complication of surgery, clinicians should anticipate local bleeding and try to avoid excessive tumour manipulation during biopsy or excision.<sup>64,66</sup> Fortunately, uncontrollable bleeding is rarely a problem during the surgical excision of MCTs.<sup>69</sup> Gastrointestinal ulceration due to MCTs can cause anorexia, vomiting, haematochezia, melaena, anaemia, abdominal pain and, in some cases, intestinal perforation and peritonitis.<sup>66,69,84,87</sup> Such complications are usually restricted to aggressive high-grade or extensive MCTs.<sup>66,69,87</sup> Nevertheless, gastrointestinal ulceration is found in about 35% to 83% of dogs with MCTs at necropsy examination.<sup>101</sup> The pathogenesis of gastrointestinal ulceration associated with MCTs appears to be caused by a combination of vascular damage, excessive gastric acid production through stimulation of H<sub>2</sub> receptors on parietal cells and hypermotility.<sup>64,66,102</sup> Malignant MCs contain 25 to 50 times more histamine than normal MCs,<sup>103</sup> and dogs with MCTs have significantly higher plasma histamine and lower gastrin levels than normal dogs.<sup>102</sup> The lower gastrin levels are thought to be the result of negative feedback from increased gastric acidity.<sup>102</sup> Plasma histamine and gastrin concentrations are not related to MCT stage, grade or tumour size, limiting their clinical usefulness in the diagnosis and prognosis of canine MCTs.<sup>66,104</sup> Acute, potentially life-threatening anaphylactic shock by sudden, massive release of histamine from neoplastic cells is very unusual, but episodes of collapse may be seen in dogs with extensive disease<sup>66,67,87</sup> and can be an uncommon complication of tumour manipulation during surgery, especially if cryosurgery or hyperthermia are employed.<sup>67,103</sup>

### Clinical prognostic factors

No single factor accurately predicts the biological behaviour or response to treatment in dogs affected by MCTs.<sup>97</sup> Clinical factors that can influence the outcome include location,<sup>60,64,66,71</sup> clinical appearance of the tumour,<sup>66,71,89,92,105</sup> growth rate,<sup>66,89,92</sup> size,<sup>89</sup> presence of systemic paraneoplastic signs,<sup>60,66,72,74,89</sup> breed,<sup>68,70,72,79–83,88</sup> sex<sup>85</sup> and clinical stage.<sup>66,85,92</sup> However, the single most valuable factor is histological grade.<sup>64</sup>

### Tumour location

Tumours in the nail bed, oral cavity, muzzle or inguinal, preputial, perineal and mucocutaneous areas are often

correlated with a worse prognosis than those in other parts of the body,<sup>60,64,66,71,74,82,106,107</sup> although this is not supported by all studies.<sup>93,108</sup> In addition, survival of dogs with incompletely resected MCTs that were treated with radiation therapy was greater for tumours located on the extremities than of those located on the trunk.<sup>109</sup>

### Tumour appearance

Local tumour ulceration, erythema or pruritus have been associated with poorer prognosis.<sup>89,105</sup>

### Systemic signs

Systemic signs, such as anorexia, vomiting, melaena, widespread erythema, oedema and gastrointestinal ulceration are most frequently associated with visceral forms of MCT and are associated with a poorer prognosis.<sup>60,66,74,89,109</sup>

### Breed predilection

Boxers are presented at significantly younger ages with MCTs than other breeds,<sup>82,88</sup> and more commonly develop histologically low- or intermediate-grade MCTs with a more favourable prognosis.<sup>72,88</sup> Although shar-peis also develop MCTs at an early age (4-year-old on average), these are frequently aggressive tumours.<sup>83,110</sup> MCTs in Labrador retrievers are also frequently more aggressive than in other breeds.<sup>83,110</sup>

### Growth rate and size

The growth rate of MCTs (tumour volume divided by the duration of time the tumour has been present) is a significant prognostic factor.<sup>92</sup> Indeed, MCTs that remain localized and are present for prolonged periods (months or years) without significant change are usually benign, and those present for over 7 months carry a favourable prognosis.<sup>92</sup> Furthermore, large tumours may be associated with a poorer prognosis.<sup>89</sup>

### Gender

Only one study reported gender-associated differences in prognosis with female dogs doing better than males following treatment of MCTs with chemotherapy.<sup>85</sup>

### Clinical stage

A clinical stage of 0 or I (Table 1) carries a better prognosis than any higher stage disease,<sup>85,92,109</sup> and metastasis at the time of initial diagnosis significantly shortens survival.<sup>93</sup> However, recent reports show that stage II MCTs can be treated successfully resulting in comparable disease-free survival time to stage 0 disease.<sup>111,112</sup> In addition, it has been shown that dogs with multiple MCTs treated surgically had a low metastatic rate and a good prognosis for a long-term survival.<sup>113</sup> Postsurgical recurrence is thought to be associated with a poorer prognosis.<sup>60,105</sup> These data suggest that with appropriate therapy, the clinical stage has minor prognostic value.

### Metastatic disease

MCTs are always considered potentially malignant,<sup>29,67</sup> but the true metastatic potential of MCTs is unknown.<sup>66</sup> Well-differentiated tumours have a metastatic rate of less than 10%, and intermediate tumours are considered low

**Table 1.** 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 a. Without systemic signs b. With systemic signs
I	One tumour confined to the dermis, without regional lymph node involvement a. Without systemic signs b. With systemic signs
II	One tumour confined to the dermis, with regional lymph node involvement a. Without systemic signs b. With systemic signs
III	Multiple dermal tumours; large infiltrating tumours with or without regional lymph node involvement a. Without systemic signs b. With systemic signs
IV	Any tumour with distant metastasis or recurrence with metastasis (including blood or bone marrow involvement)

to moderate in metastatic potential.<sup>66,108</sup> In addition to greater local infiltration, undifferentiated tumours have a much higher metastatic rate, ranging from 55% to 96%.<sup>67,92,114</sup> Differences in reported metastatic rates for different histological grades may be in part due to the somewhat subjective system of grading.<sup>66</sup>

Most MCTs first metastasize to local lymph nodes and then to the spleen (46%), liver (41%) or other visceral organs.<sup>29,66,73,74</sup> Lung involvement is very uncommon.<sup>66</sup> Neoplastic MCs may be observed in the bone marrow and in the peripheral blood in cases of widespread systemic dissemination.<sup>66</sup> The visceral form of a MCT (disseminated mastocytosis) is usually preceded by an undifferentiated primary cutaneous MCT.<sup>60,66,67</sup>

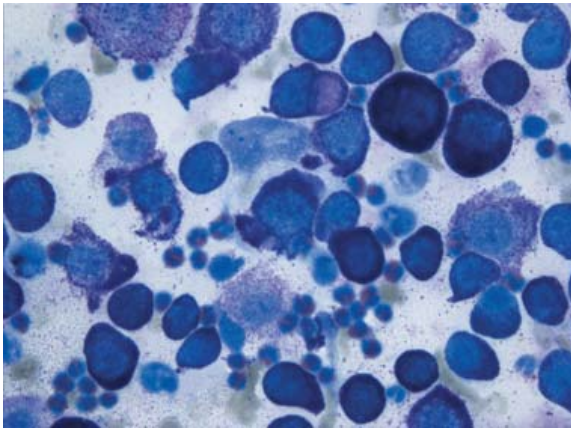
### Diagnostic workup

The diagnostic evaluation of dogs with suspected MCTs has three goals: (i) definitive diagnosis by cytology and/or histopathology, (ii) clinical staging, and (iii) documentation of paraneoplastic clinical signs.<sup>68,115</sup>

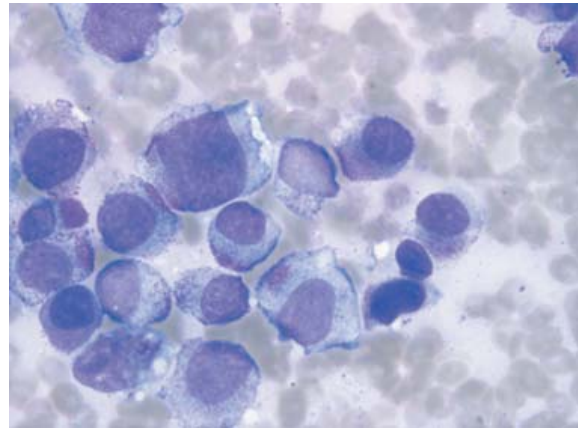
### Tumour cytology

Fine-needle aspirates (FNAs) of dermal and subcutaneous masses are a simple technique and should be performed prior to surgery because a preoperative diagnosis of MCTs influences the type and extent of surgical intervention.<sup>49,60,68,71</sup> MCTs can generally be diagnosed by cytological examination of stained smears of FNAs.<sup>49,66,74,87,107</sup> Indeed, FNA yields a correct diagnosis in 92% to 96% of histologically confirmed canine MCTs.<sup>66,82</sup> Cytology of MCTs reveals a discrete round-cell population with moderate amounts of cytoplasm containing purplish red cytoplasmic granules of variable number and size.<sup>60,68,71,72,103</sup> The cells have a round to oval nucleus that may be masked by intense staining in highly granulated cells (Fig. 2). Other cells found on cytology are variable numbers of eosinophils and/or plump spindle cells, presumably fibroblasts. MC granules can be stained with routine haematological stains (Wright's, Giemsa, Leishman, etc.) as well as rapid modified





**Figure 2.** Fine-needle aspirate smear of a mast cell tumour showing mainly highly granular mast cells: staining is so intense that cellular morphology is often obscured (May–Grünwald Giemsa).



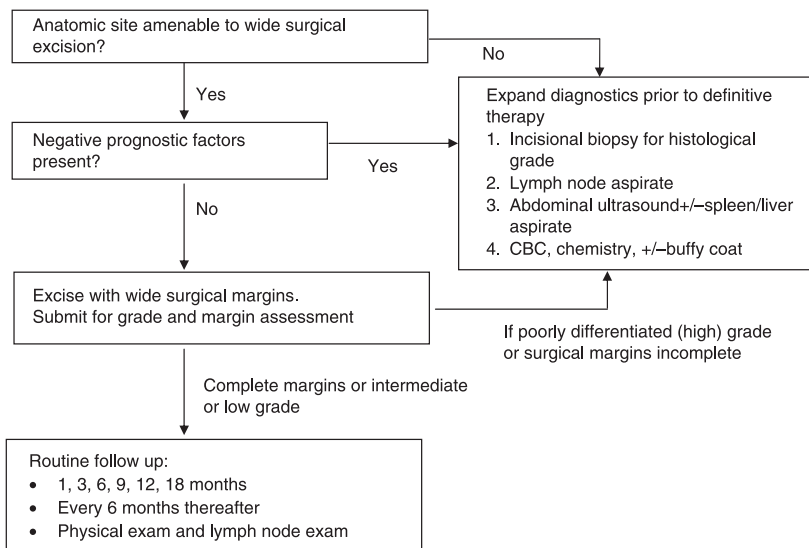
**Figure 3.** Aspirate smear from a mast cell tumour showing a highly cellular preparation of poorly granular mast cells with several nucleoli, moderate to marked variation in cell and nuclear size, and many mitotic figures (May–Grünwald Giemsa).

Romanowsky-type stains (e.g. Diff-Quik™) used in most practices.<sup>116</sup> However, granules in some MCTs stain poorly with rapid stains unless the slide is immersed for several minutes in the alcohol fixative.<sup>66,103,116</sup> In addition, poorly granulated mast cells may be difficult to recognize (Fig. 3).<sup>66,68,74</sup> Although grading of MCTs requires histological evaluation, cell morphology and staining characteristics observed on cytological preparations give an indication about the degree of differentiation.<sup>68,103</sup> Anaplastic or high-grade tumours normally yield a highly cellular sample with cells having a low number of cytoplasmic granules, irregular nuclei and increased pleomorphism and mitotic figures.<sup>74,117</sup> In some cases, poorly granulated cells of anaplastic tumours may be more easily recognized in cytological smears and imprints than in histological sections due to the greater resolution of granules in cytology.<sup>103</sup>

**Further diagnostic workup**

The extent of ancillary diagnostic workup and staging following a cytological diagnosis of MCT has recently been

re-evaluated and depends on the presence or absence of negative prognostic factors<sup>60,66</sup> as illustrated by Thamm and Vail (Fig. 4).<sup>66</sup> The minimum workup in cases requiring presurgical staging consists in FNA of the draining lymph nodes even if they are normal in size<sup>107</sup> and abdominal ultrasound.<sup>60,66</sup> Evaluation of the sublumbar lymph nodes may require ultrasonography or other imaging techniques.<sup>67</sup> If enlargement of the sublumbar nodes is present, metastatic disease should be presumed.<sup>67</sup> The interpretation of lymph node cytology is not always easy because MCs can be found in lymph node aspirates in normal animals.<sup>68,87</sup> The WHO guidelines suggesting that cytological specimens need only be evaluated for the presence or absence of MCs may therefore lead to an incorrect diagnosis of metastasis.<sup>118</sup> Current recommendations are that metastasis to a lymph node should be diagnosed by cytology if MCs represent more than 3% of the cell population in the aspirate.<sup>87</sup> However, even with this criterion, up to 25% of normal dogs would be diagnosed with a metastatic MCT,



**Figure 4.** Suggested diagnostic steps for canine cutaneous mast cell tumours.

Adapted from Thamm & Vail<sup>66</sup>

and evaluation of the degree of cytological differentiation and clustering or aggregation of cells should also be considered in the final cytological diagnosis of metastatic disease.<sup>66,68,87</sup> In suspicious cases, surgical extirpation of a lymph node and histological assessment should be considered.<sup>66</sup>

Knowledge of the extent of gross MCT margins allows more appropriate planning for surgery or radiotherapy and the exact extent of local tumour margins was upgraded in 19% of cases with ultrasound and in 65% of cases with computed tomography in one study.<sup>119</sup> However, it has to be considered that such examinations add additional costs and therefore may only be justified if the tumour location does not allow wide excisional margins.<sup>66,118</sup>

Thoracic radiographs are seldom indicated as MCTs very rarely metastasize to the lungs. Moreover, when lung metastasis does occur, diffuse interstitial infiltrates are more common than discrete nodules.<sup>66,103</sup> Nevertheless, thoracic radiographs are reasonable in older patients to rule out an unrelated disease that may complicate anaesthesia if an expensive or invasive procedure is necessary.<sup>66</sup>

Aspiration cytology of sonographically suspicious lesions in the liver or spleen is recommended but interpretation can be complicated by the presence of nonmalignant MCs in these organs in normal dogs.<sup>66</sup>

The validity of screening buffy coat smears in dogs with cutaneous MCTs for possible systemic involvement has been disputed because mastocytosis is more frequently observed in dogs with inflammatory conditions than MCTs.<sup>66,120</sup> Moreover, the visceral form of MCTs constitutes a small minority of all canine cases and many authors have abandoned the routine examination of buffy coat smears, reserving this diagnostic procedure for selected cases.<sup>60,66,87,97</sup>

Equally, bone marrow aspiration is no longer recommended in the routine staging of canine MCTs.<sup>66</sup> The vast majority of dogs with cutaneous MCTs do not have circulating MCs or evidence of bone marrow infiltration<sup>66,87</sup> and involvement of peripheral blood or bone marrow is unlikely in the absence of disease in the regional lymph nodes or abdominal organs.<sup>66,87</sup> Moreover, visceral MCTs carries a very grave prognosis and the additional presence or absence of neoplastic MCs in blood or bone marrow rarely alters treatment decisions.

### Clinical staging

The WHO clinical staging system for MCTs<sup>71</sup> (Table 1) is widely used together with the histological grade in the aid of prognosis, therapy selection and in clinical trials.<sup>60,66–68,86,87</sup> However, recent studies have questioned the utility of this system, especially with respect to clinical stage III tumours.<sup>83,105,121</sup> In general, clinical stage 0 and I tumours have a better prognosis than higher stage disease.<sup>85,92</sup> According to the WHO staging system multiple cutaneous nodules and large infiltrating tumours are staged identically as stage III, although several studies suggest that no difference in outcome exists between patients with single MCTs and those with multiple MCTs of the same histological grade.<sup>83,89,122</sup> Many authors therefore suggest that the category of 'multiple tumours' should be removed from the stage III grouping, and each

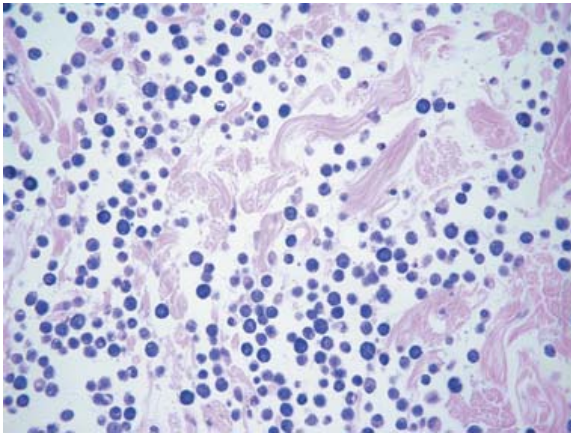
tumour should be staged individually in dogs with multiple tumours.<sup>66,68,87</sup> Second, the term 'infiltrating' is misleading since according to the histological grading system for MCTs of Patnaik *et al.*,<sup>81</sup> all grade II and III tumours infiltrate the subcutis and therefore would become clinical stage III, although the prognosis of some grade II tumours is fairly good.<sup>87</sup> A preliminary study suggests that some subcutaneous MCTs even have a slightly better long-term prognosis than higher grade dermal variants.<sup>123</sup> As a consequence of the above-mentioned weaknesses, a significant number of dogs are probably treated more aggressively than necessary.<sup>87</sup> Therefore, it is necessary to update the WHO clinical staging system for cutaneous MCTs but so far, no consensus exists over the best staging procedure for canine MCTs and various authors use their own systems.<sup>68,97</sup>

### Histological findings and staining properties

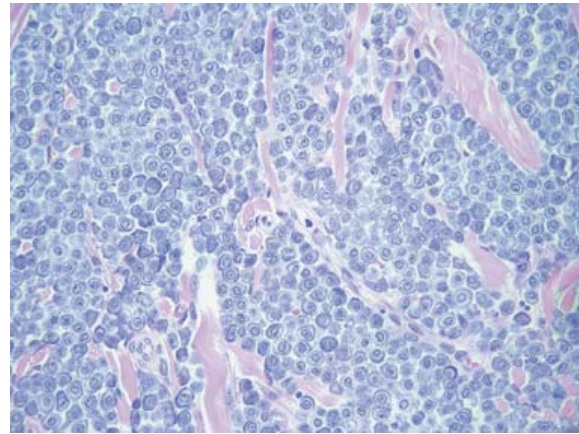
Although FNA cytology is convenient for diagnosis, it does not provide a histological grade and excisional samples should be submitted for histological grading and margin assessment.<sup>29,66,68,87,97</sup> The diagnosis of MCTs is straightforward in most cases. They present as nonencapsulated dermal and/or subcutaneous infiltrative growing masses composed of round cells arranged in densely packed cords or loose sheets. Cellular morphology depends on the degree of differentiation but most neoplastic MCs have a distinct cell membrane, a central round nucleus and variable numbers of cytoplasmic granules that stain light-grey blue with haematoxylin and eosin or purple with metachromatic stains such as toluidine blue. The collagenous stroma varies from scant to abundant, and may appear oedematous or hyalinized. Variable numbers of eosinophils are either dispersed throughout the neoplastic mass or form aggregates. Marked deposition of mucin and perivascular hyalinization may be seen in response to the eosinophilic infiltration, and eosinophilic vasculitis may be present. Foci of marked eosinophilic degranulation may entrap brightly eosinophilic collagen bundles to form flame figures.

Well-differentiated MCTs (Fig. 5) consist of fairly well-circumscribed but nonencapsulated masses composed of round cells with a central small nucleus, a single inconspicuous nucleolus and abundant, highly granulated cytoplasm. Cytoplasmic borders are distinct, cellular variation is rare and mitotic figures are almost never seen. Tumour cells are arranged in rows or loose sheets separated by collagen bundles. The overlying epidermis is usually intact. These tumours are confined to the interfollicular dermis in the Patnaik grading system (Table 2); however, in the authors' experience and of others, tumours with the above described morphology may also invade the subcutaneous tissue.<sup>29,124</sup>

Intermediate-grade MCTs (Fig. 6) are not as well circumscribed and are comprised of closely packed cells with distinct cell borders, an increased nuclear-to cytoplasmic ratio and fewer granules than a well-differentiated tumour. Giant, binucleate or occasional spindle-shaped cells may be present. Small numbers of mitotic figures are seen. The tumour cells are arranged in groups separated by a collagenous stroma that may be thick or hyalinized with areas of oedema and necrosis.



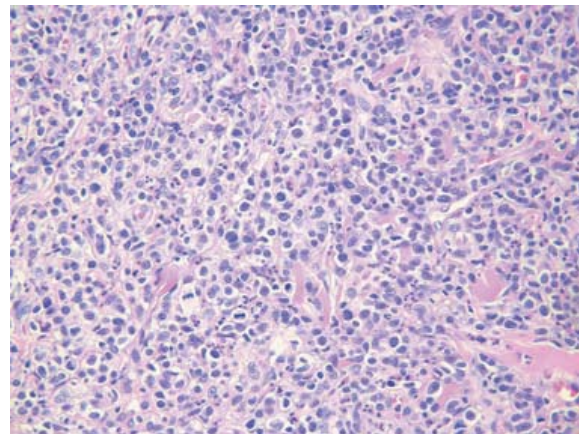
**Figure 5.** Well-differentiated mast cell tumour. Note that tumour cells are loosely arranged and separated by collagen bundles. Cells have a well granulated cytoplasm and distinct cell borders. There is little cellular pleomorphism. Haematoxylin and eosin,  $\times 400$ .



**Figure 6.** Intermediate-differentiated mast cell tumour. Note the closely packed cells which have a finely granulated cytoplasm and distinct cell borders. Nuclear pleomorphism is moderate and mitotic figures are rare. Haematoxylin and eosin,  $\times 400$ .

Undifferentiated (anaplastic) MCTs (Fig. 7) are poorly circumscribed and the majority of them consist of poorly granulated round to polygonal highly pleomorphic cells. The nucleus is large, vesicular and irregular in shape and has several nucleoli. Bi- or multinucleated cells are present and mitotic figures are numerous. The tumour cells infiltrate the subcutis and deeper tissues. Necrosis and ulceration are common secondary features.<sup>29,30,125</sup>

Cellular density and morphology varies significantly between grades, and poorly differentiated MCTs can be confused with other round-cell tumours. Thus, numerous attempts have been undertaken to apply histochemical and immunohistochemical stains for a definitive diagnosis of poorly differentiated MCTs. In one study that examined the use of the histochemical stains, alcian blue pH 2.5, azure and eosin, Giemsa, Luna's method for MCs, periodic acid-Schiff reaction, safranin O, toluidine blue, Torren's method, Unna's method, chloracetate esterase, and the affinity stains, avidin peroxidase, wheat germ agglutinin, and concavalin-A agglutinin, as well as immunohistochemical stains with CD68, none of the staining techniques was found to be ideal.<sup>126</sup> Based on a previous publication and our own experience, immunohistochemistry with antitryptase antibodies or histochemical stains for chymase activity may, however, be helpful in the diagnosis of poorly granulated MCTs.<sup>127</sup> Indeed, seven of seven (100%) grade I, six of seven (85.7%) grade II, and three of seven (42.9%) grade III tumours were diagnosed as MCTs based on these stains.<sup>127</sup> In addition, MCTs are positive for the common



**Figure 7.** Poorly differentiated mast cell tumour. Note the pleomorphic non-granulated cells, the variably sized and shaped nuclei with a prominent nucleolus and the high number atypical mitotic figures. Intermingled with the tumour cells are numerous eosinophilic granulocytes. Haematoxylin and eosin,  $\times 400$ .

leucocyte marker. CD45, and retain variable expression of the leucocyte antigens CD45RA and CD18.<sup>29</sup> As discussed earlier, MCTs also express KIT, but none of these antigens is specific for MCTs. Very recently, the production and characterization of mouse monoclonal antibodies specific for canine MCTs were reported.<sup>37</sup> In this study, one of the antibodies (MAb 9-3; IgM) recognized a 74-kDa protein on

**Table 2.** Histological grading of mast cell tumours suggested by Patnaik *et al.* 1984<sup>81</sup>

Histological grade	I	II	III
Extent of tumour	Dermis	Dermis and subcutis	Invades subcutis and deep tissues
Cellularity	Mast cells separated by collagen fibres	Moderately to highly cellular	Highly cellular
Cellular morphology	Round, monomorphic, distinct cell borders, medium-sized granules	Round to ovoid, moderately pleomorphic cells with scattered spindle and giant cells, distinct cell borders, fine granules	Pleomorphic, medium-sized, round, ovoid or spindle-shaped cells, indistinct cell borders, fine granules or none
Nuclear morphology	Round	Round to indented, single nucleoli, some cells with double nuclei	Indented and vesiculated nuclei with one or more nucleoli, scattered multinucleated cells



the surface and the cytoplasmic granules on all eight MCTs investigated but not on normal tissue MCs. A second antibody (MAb 80; IgM) recognized a 167-kDa and a 248-kDa protein on both neoplastic and normal tissue MCs. Further studies with larger numbers of tumours will show if MAb 9-3 proves to be a suitable marker for the diagnosis of poorly differentiated canine MCTs. For the present, however, a final diagnosis of undifferentiated MCTs remains difficult and may require the use of multiple antibodies to exclude other poorly differentiated round-cell tumours.

### Grading based on morphology

Three histopathological classifications have been proposed to identify the degree of differentiation of canine MCTs.<sup>61,81,92</sup> All grading systems differentiate between well-differentiated (low grade, mature), intermediate-grade, and undifferentiated (poorly differentiated, anaplastic, high grade) MCTs. Among them, the classification proposed by Patnaik *et al.*<sup>81</sup> (Table 2) is considered to be the most complete and is the most frequently used system.<sup>46,47,51,128–138</sup> Whereas Hottendorf and Nielsen did not assign any numbers to the grade of differentiation, well-differentiated tumours are considered as grade I by the Patnaik system but grade III by the system proposed by Bostock.<sup>61,81,92</sup> To avoid confusion, the terms well differentiated, intermediate and poorly differentiated are frequently employed instead of grade numbers, especially if the grading system used is not explicitly reported. Numerous studies have shown significant differences between well- and poorly differentiated MCTs in survival times.<sup>81,92,126,128,131,139</sup> However, histological grading is of limited prognostic value for tumours of intermediate differentiation (more than 40% of all canine MCTs) as these tumours behave unpredictably in a benign manner, recur or metastasize.<sup>118,140</sup> In addition, studies have shown variation between pathologists using identical grading systems in their assignment of histological grades for MCTs.<sup>141,142</sup> This is mainly due to the fact that histological grading is based on the use of subjective parameters such as invasiveness, cellularity, and cellular morphology.<sup>130,141–143</sup> As a result, various attempts have been undertaken to use more objective methods to assess the grade of MCTs.

Nuclear morphometry has been shown to be significantly associated with histological grading of various tumours in humans and animals.<sup>134,144–148</sup> Two groups investigated nuclear morphological features as possible indicators of histological grade in canine MCTs.<sup>130,149</sup> These groups demonstrated significant differences between grades I and III and grades II and III for mean nuclear area, nuclear diameter and nuclear perimeter but differences between grades I and II were not significant (Table 3). The mitotic index (MI) has been shown to be a strong predictor of outcome for a variety of human and canine cancers<sup>150,151</sup> and several studies have demonstrated a prognostic value of MI in canine MCTs (Table 3).<sup>124,130,135–137,143,149</sup> Moreover, in one recent study, the median survival for dogs with a MI  $\leq 5$  was significantly longer (70 months) than for those with a MI  $> 5$  (2 months), regardless of histological grade.<sup>137</sup> However, in the authors' experience, MI may be difficult to assess in highly granulated MCTs. The results of the studies undertaken to give a prognostic indicator with

different morphological features and morphometric variables are summarized in Table 3. In conclusion, morphological features are useful in predicting MCT behaviour but predicting prognosis in intermediate-grade MCTs still remains problematic. The MI seems to be a strong predictor for the prognosis with regard to survival time. However, in the two studies in which the impact of MI on the rate of recurrence was investigated, the results are controversial.<sup>136,143</sup> Nevertheless, the MI is an objective measure and may help to limit interobserver variation in the histological evaluation of MCTs. A problem that remains to this date is that the various studies used different cut-off values and thus the data are still difficult to apply in a diagnostic setting.

### Grading based on proliferation markers

Uncontrolled cellular proliferation is a hallmark of cancer and, as such, measures of cellular proliferation have been extensively used in an attempt to predict behaviour in neoplastic disease.<sup>47,124,126,131,133,138,143,152–158</sup> In veterinary medicine, the most commonly used methods include staining for argyrophilic nucleolar organizer regions (AgNORs), immunohistochemistry proliferating cell nuclear antigen (PCNA) and for Ki67, all of which have been investigated in canine MCTs (Table 3).<sup>156,159</sup>

AgNORs are areas in the nucleus that are associated with proteins, such as nucleolin and nucleoplasmin substructures, involved in ribosomal RNA transcription.<sup>160</sup> They are widely used as a marker of tumour kinetics and tumour metabolic activity. AgNORs bind silver molecules and can be visualized by light microscopy using a silver-based histochemical stain.<sup>161</sup> The quantity of AgNORs per nucleus has been shown to be proportional to the rate of cell proliferation or cell doubling time *in vitro*<sup>162–164</sup> and the rate of tumour growth *in vivo*.<sup>165–167</sup> A number of studies have shown that higher AgNOR counts in MCTs are associated with increased mortality, local recurrence and metastasis.<sup>47,124,138,143</sup>

PCNA is a protein that interacts with DNA polymerases, acting as an auxiliary factor for DNA replication and repair.<sup>168–170</sup> Although PCNA has an extended half-life and is involved in multiple nuclear functions, maximal PCNA expression is commonly seen in the synthesis phase of the cell cycle (S-phase).<sup>49,171,172</sup> Two studies have shown an association between PCNA expression and increased mortality in canine MCTs.<sup>47,143</sup> However, only one of these two studies found an association between high PCNA expression and recurring or metastatic tumours<sup>47,143</sup> and other studies found no correlation between PCNA expression and prognosis.<sup>131,138</sup> Furthermore, PCNA expression has not been found to be a prognostic marker independent of the histological grade in any study.<sup>47,126,131,138,143</sup>

Ki67 is a nuclear protein that is expressed in all active phases of the cell cycle but is not present in noncycling cells.<sup>173,174</sup> The relative number of Ki67-positive cells is used to determine the proliferation index or the relative number of cells actively involved in the cell cycle (growth fraction).<sup>156,159,173,174</sup> A high Ki67 expression in MCTs has been shown to be associated with increased mortality, the rate of local recurrence and metastasis.<sup>47,131,138</sup> In addition, Ki67 was found to be better than AgNORs for identifying MCTs associated with decreased survival,



**Table 3.** Summary of published evidence of prognostic indicators for canine cutaneous mast cell tumour

Prognostic indicator	Outcome			Mortality	Total no. of cases	Authors and follow-up period
	Local recurrence	Metastasis	Survival time			
<b>Histological grade</b>						
Well	–	–	51 weeks	12%	114	Bostock 1973 <sup>92</sup>
Intermediate	–	–	28 weeks	17%		2.5 years
Poor	–	–	18 weeks	77%		
Well	–	–	Alive after study: 83%	7%	83	Patnaik <i>et al.</i> 1984 <sup>81</sup>
Intermediate	–	–	Alive after study: 44%	56%		1500 days
Poor	–	–	Alive after study: 6%	94%		
Well	–	–	Alive after 20 months: 100%	–	75	Magnol, Toulemonde, 1987 <sup>139</sup>
Intermediate	–	–	Alive after 20 months: 65%	–		
Poor	–	–	Alive after 20 months: 12%	–		
Well	–	–	–	9%	90	Simoes <i>et al.</i> 1994 <sup>126</sup>
Intermediate	–	–	–	29%		601 days
Poor	–	–	–	58%		
Well	–	–	Alive after 24 months: 17%	–	120	Abadie <i>et al.</i> 1999 <sup>131</sup>
Intermediate	–	–	Alive after 20 months: 16%	–		Between 12 and 24 months
Poor	–	–	Alive after 20 months: 1%	–		
Poor	–	–	1 month	–	53	Ginn <i>et al.</i> 2000 <sup>128</sup>
<b>Invasiveness</b>						
	Increased	–	–	Increased	32	Preziosi <i>et al.</i> 2004 <sup>135</sup>
	Increased	–	–	Increased	32	Preziosi <i>et al.</i> 2007 <sup>136</sup>
<b>Mitotic index</b>						
0–10‡	–	–	40 weeks	–	50	Bostock <i>et al.</i> 1989 <sup>124</sup>
> 10‡	–	–	11 weeks	Increased		40 weeks
9.4§	Yes	–	–	–	122	Simoes <i>et al.</i> 1994 <sup>126</sup>
5.5§	No	–	–	–		
High (no cut-off values)	–	–	–	Increased	32	Preziosi <i>et al.</i> 2004 <sup>135</sup>
High (no cut-off values)	Not increased	–	–	Increased	32	Preziosi <i>et al.</i> 2007 <sup>136</sup>
> 5‡	–	–	2 months (5 months for grade II)	–	148	Romansik <i>et al.</i> 2007 <sup>137</sup>
≤ 5‡	–	–	70 months	–		
<b>Nuclear morphology</b>						
	Significant difference between grade I and III and II and III			–	24	Strefezzi <i>et al.</i> 2003 <sup>130</sup>
	Significant difference between grade I and III and II and III			–	35	Maiolino <i>et al.</i> 2005 <sup>149</sup>
<b>AgNOR</b>						
≥ 4 (≥ 4.9 for mortality)	–	–	17 weeks	Increased	50	Bostock <i>et al.</i> 1989 <sup>124</sup>
< 4	–	–	50 weeks	–		40 weeks
2.9 (recurrence)	Yes	Yes	–	Increased	122	Simoes <i>et al.</i> 1994 <sup>126</sup>
2.8 (metastasis)	–	–	–	–		
> 2.25 (mortality)	–	–	–	–		
2.2 (recurrence)	No	No	–	–		
2.3 (metastasis)	–	–	–	–		
> 1.8	–	–	–	Increased	121	Scase <i>et al.</i> 2006 <sup>138</sup>
Increased	Increased	Increased	–	Increased	56	Webster <i>et al.</i> 2007 <sup>47</sup>
<b>Ki67</b>						
> 1%	–	–	–	Increased	121	Scase <i>et al.</i> 2006 <sup>138</sup>
> 1.8 % for grade II	–	–	–	Increased	85	
< 55/1000 cells	–	–	–	–	120	Abadie <i>et al.</i> 1999 <sup>131</sup>
≥ 55 < 135/1000 cells	–	–	–	Increased		
≥ 135/1000 cells	–	–	–	increased		
≥ 93/1000 cells for grade II	–	–	–	increased		
Increased	Increased	Increased	–	Increased	56	Webster <i>et al.</i> 2007 <sup>47</sup>
> 23/1 cm <sup>2</sup>	Increased	Increased	< 24 months	Increased		
≤ 23/1 cm <sup>2</sup>	–	–	> 24 months	–		
<b>Ki67 × AgNOR</b>						
≥ 54	Increased	Not significant	< 24 months	Increased	56	Webster <i>et al.</i> 2007 <sup>47</sup>
< 54	–	Not significant	> 24 months	–		
<b>PCNA</b>						
393.1 (recurrence)	No	No	–	–	122	Simoes <i>et al.</i> 1994 <sup>126</sup>
451.2 (metastasis)†	–	–	–	–		
875.8 (recurrence)	Yes	Yes	–	Increased		
976.7 (metastasis)	–	–	–	–		
> 261 (mortality)†	–	–	–	–		
Increased‡	Not significant	Not significant	–	Increased	56	Webster <i>et al.</i> 2007 <sup>47</sup>

Table 3. Continued

Prognostic indicator	Outcome			Mortality	Total no. of cases	Authors and follow-up period
	Local recurrence	Metastasis	Survival time			
<b>KIT staining pattern</b>						
II and III	Increased	–	–	Increased	98	Kiupel <i>et al.</i> 2004 <sup>46</sup>
Yes	Mutations associated with grade II and III MCTs				88	Zemke <i>et al.</i> 2002 <sup>51</sup>
Yes	Increased	Increased	–	Increased	60	Webster <i>et al.</i> 2006 <sup>54</sup>
<b>IMVD</b>						
> 14.1 mm <sup>-2</sup>	6–7 months¶	6–7 months¶	–	Increased	32	Preziosi <i>et al.</i> 2004 <sup>135</sup>
< 14.1 mm <sup>-2</sup>						

AgNOR: argyrophilic nucleolar organizing region counts/cell; Ki 67: values are given as % of positive nuclei counted ; positive cells per 1000 cells; positive cells/1 mm × 1 mm grid area; \*PCNA: proliferating cell nuclear antigen/1 mm × 1 mm grid area; †per 5/HPF; KIT staining pattern: (I) membrane-associated, (II) focal stippled cytoplasmic, (III) diffuse cytoplasmic; IMVD: intratumoural microvessel density mm<sup>-2</sup>; ¶If grades are mentioned the Patnaik grading system was used; ‡Number of mitotic figures/10 high power fields (400×); §Number of mitotic figures per 1000 cells; ¶No information given if local or distant recurrence; – No data presented, not significant or no statistical analysis given.

although AgNORs were better markers for identifying MCTs with a decreased disease-free interval.<sup>47</sup> Importantly, Ki67 has been shown to be a prognostic factor that is independent of histological grade.<sup>47,138</sup> Thus, Ki67 immunohistochemistry can be used as an objective prognostic marker in MCTs.

KIT expression was found to be significantly correlated to histological grade, Ki67 expression, AgNORs and tumoural necrosis in one study.<sup>133</sup> Furthermore, significant correlations were also established between Ki67 and AgNORs with histological grade, tumour necrosis and epidermal ulceration. In this study, no differences were observed between focal and diffuse cytoplasmic KIT staining and histological variables.<sup>133</sup> The latter finding is in contrast to results presented in other studies, which demonstrated an association between aberrant cytoplasmic KIT expression and either increased proliferation index, the presence of *c-kit* mutations, increased local recurrence or decreased survival.<sup>45–47,51</sup>

Tumour angiogenesis plays a key role in tumour growth and metastasis<sup>175,176</sup> and the prognostic role of intratumoural microvessel density (IMVD) has been demonstrated for canine mammary tumours and squamous cell carcinoma.<sup>177–180</sup> IMVD has also been evaluated by immunohistochemical staining of canine MCTs for the endothelial marker factor VIII (von Willebrand's factor)-related antigen, and found to be associated with tumour recurrence and mortality.<sup>135</sup>

Abnormal DNA content (aneuploidy) was investigated in 40 canine MCTs using flow cytometry.<sup>132</sup> In this study, over 70% of the MCTs were diploid. When comparing diploid and aneuploid tumours, a significant difference was found between clinical stage I versus non-stage I tumours but only a trend towards shorter survival was found in dogs with aneuploid tumours.<sup>132</sup>

Mutation of the p53 gene is the most common genetic alteration known to occur in human cancer.<sup>181</sup> The product of the p53 tumour-suppressor gene is a nuclear transcription factor whose functions include cell cycle arrest in cells with damaged DNA, induction of transcription of DNA repair enzymes, and the induction of apoptosis in cells that have sustained irreparable genetic damage.<sup>181–184</sup> Two studies investigated p53 immunoreactivity in canine MCTs but neither could show an association with survival or tumour recurrence.<sup>128,129</sup>

Similarly, expression of survivin, an inhibitor of apoptosis, was investigated in canine MCTs but no association was found between survivin expression and survival.<sup>138</sup>

Matrix metalloproteinases have been associated with tumour invasion and metastasis in a variety of neoplasms in humans and animals.<sup>185–187</sup> Matrix metalloproteinases 2 and 9 are secreted by canine MCTs and activated by exocytosed chymase.<sup>68,204</sup> Their expression in MCs is regulated by KIT ligand and tumour growth factor-β.<sup>188,189</sup> Moreover, undifferentiated MCTs were found to express higher levels of these enzymes than intermediate-grade MCTs.<sup>190</sup>

Vascular endothelial growth factor (VEGF) has also been investigated as a mediator of disease-related angiogenesis and potential autocrine growth regulator of neoplastic cells.<sup>191–194</sup> Although canine MCTs were found to express both VEGF and VEGF receptors, VEGF was not found to be a growth regulator in canine MCTs.<sup>195</sup>

The results of all the studies investigating proliferation markers as prognostic factors are summarized in Table 3. From these studies it can be concluded that Ki67 and AgNORs seem to be the most valuable factors predicting MCT behaviour. Of these, the Ki67 index is much easier to assess and is a factor predictive of prognosis independent of Patnaik grading. However, the various studies investigating Ki67 have used different cut-off values to differentiate between tumours that are likely to have a favourable prognosis and those that are likely to have a poor prognosis, making practical widespread application of a Ki67 index difficult. Thus, the MI that can be assessed much easier in a diagnostic setting seems to be equally valuable.

### Treatment of canine cutaneous mast cell tumours

Treatment decisions are based on the clinical presentation of the disease and on the presence or absence of prognostic factors. There are numerous publications that evaluate various treatments and combinations of those. Unfortunately, many of these reported studies were done in a retrospective way, based on a small number of cases, lacked appropriate concurrent control groups and have included MCTs of various clinical grades and stages necessitating careful interpretation of the findings. The following section is a more practical approach to treatment options and appropriate management of this disease.

### Well-differentiated to intermediate-grade MCTs

The management of well-differentiated (grade I, low grade) and intermediate-grade MCTs for most anatomic sites consists in complete surgical resection. Various reports describe effective local tumour control in about 84% to 89% of grade II MCTs after complete surgical removal with local failure reported in 5% to 11% of the cases and distant metastasis in 5% to 22%. However, many studies report the development of new MCTs in up to 44% of the cases.<sup>89,93,196–199</sup>

The historically suggested 3 cm surgical margins<sup>200</sup> for resection of canine MCTs have recently been replaced by studies demonstrating that a 2 cm lateral margin and a deep margin of one fascial plane is sufficient for the complete excision of most grade I and II MCTs.<sup>197,201</sup> For cases with clean excision of stage I well-differentiated to intermediate-grade tumours, a rigorous re-evaluation schedule and close monitoring for recurrence rather than adjuvant therapy is recommended (Fig. 6).<sup>66</sup> Presurgical consolidation and reduction of tumour burden with prednisone can be realized in 70% and will improve the likelihood of obtaining complete resection margins.<sup>121,202</sup>

In a recent report of incompletely excised grade II MCTs, 23% of all tumours recurred.<sup>199</sup> Adjuvant therapy with radiation is known to improve local tumour control and the incorporation of further prognostic factors, such as Ki67 scores or MI may help to select patients that are most likely to profit from ancillary local therapy.<sup>137,199</sup> Tumours on the extremities, for example, can almost always be controlled with amputations, but the routine use of adjuvant radiotherapy and marginal surgical excision yields high local control rates as well as excellent functional outcome. Incompletely excised intermediate-grade MCTs in all locations, treated with adjuvant radiation therapy result in a 1- to 2-year disease-free interval (DFI) in 81% to 95% of cases.<sup>106,109,198,203,204</sup> In contrast to 'high-risk' tumours (e.g. tumours occurring at mucocutaneous junctions, grade II stage II or grade III MCTs),<sup>112</sup> prophylactic irradiation of cytological negative lymph nodes has not been found to increase overall survival.<sup>198</sup>

### Well-differentiated to intermediate-grade MCTs with lymph node involvement

For dogs with well-differentiated to intermediate-grade, solitary cutaneous tumours and metastasis to regional lymph node (stage II), several approaches have been described. Good outcomes with a median DFI of 40.6 months have been found for combined therapy with surgery and irradiation.<sup>196</sup> While adjuvant chemotherapy is also possible, the need for systemic therapy in these cases is questionable.

### High-grade or high-risk MCTs

The median survival for patients with high-risk tumours (e.g. mucocutaneous, gingival, perineal, prepuccial, nail bed), grade II stage II or grade III MCTs varies between 3.5 and 20 months after surgical excision alone and combination of surgery and radiation therapy, respectively.<sup>81,143,206,207</sup> In spite of acceptable results with surgery and radiotherapy,<sup>206</sup> the majority of veterinary oncologists feel that local therapy alone is insufficient for optimal control of high-grade MCTs.<sup>112</sup> The outcome of such cases treated with



**Figure 8.** Six-year-old Tervueren with an ulcerated mast cell tumour on the lateral nose after four weekly cycles of therapy with vinblastine/prednisolone. The tumour size was reduced by more than 50% prior to surgery, which was then possible to be performed with clean margins.

a multimodal approach aiming at adequate local control and additional use of prednisone/vinblastine chemotherapy yielded superior results with a DFI of 45 months and 65% of patients alive at 3 years (Fig. 8).<sup>205</sup> When wide surgical excision is not possible, cytoreductive surgery or palliative radiation using coarse fractions of 6–8 Gy with total doses of 24–36 Gy, followed by systemic chemotherapy with prednisone/vinblastine, may be considered.<sup>66</sup> As adequate local control is practically impossible in these cases, prognosis is only fair with median survival of approximately 5 months.<sup>105</sup>

### Metastatic or recurrent MCTs

A large number of patients not cured of their disease during initial treatment will develop local and/or loco-regional failure (5% to 23%) in the absence of distant metastatic disease.<sup>118,140,199</sup> In such cases, re-evaluation with staging is necessary and treatment should follow similar rules as for primary disease. Systemic treatment with vinblastine/prednisone in one study involving mostly incompletely or marginally resected intermediate-grade MCTs yielded low rates of local recurrence (3.7%) and metastatic disease (15%).<sup>208</sup> In addition, up to 44% of dogs cured of a previous MCT will develop *de novo* MCTs which require individual staging and grading.<sup>89,196–199</sup>

In cases of visceral metastatic disease, any attempt of local control should be accompanied by systemic therapy consisting in cytotoxic chemotherapy and ancillary therapy aiming at controlling or reducing the systemic effects of release of MCT granules. The recommended choice of chemotherapy is vinblastine/prednisone,<sup>105</sup> or the combination protocol using vinblastine/cyclophosphamide/prednisone.<sup>209</sup> CCNU (lomustine) has also been shown to result in measurable, albeit short lived response (2.6–5 months) in 42% to 47% of macroscopic MCTs.<sup>210</sup> Many oncologists are using a protocol that incorporates vinblastine/CCNU/prednisone (Table 4); however, results on the outcome of this protocol in peer-reviewed literature are still pending.

**Table 4.** Selected chemotherapy protocols for treatment of canine cutaneous mast cell tumour to be used in combination therapy aiming at adequate local control of primary and local metastatic disease

Protocols	Dose	Frequency of administration
Vinblastine (VBL)/Prednisone <sup>112,218</sup>	VBL: 2 mg/m <sup>2</sup> intravenously Prednisone: 2 mg/kg <sup>1</sup> orally 1 mg/kg <sup>1</sup> orally 1 mg/kg <sup>1</sup> orally	Once weekly for 4 weeks, followed by 4 treatments every 2 weeks  Week 1: once daily Week 2 + 3: once daily Thereafter: every other day
Vinblastine/Cyclophosphamide (CTX)/Prednisone <sup>209</sup>	VBL: 2–2.2 mg/m <sup>2</sup> intravenously CTX: 200–250 mg/m <sup>2</sup>  Prednisone: 1 mg/kg <sup>1</sup> orally	Every 3 weeks (day 1 of 21-day protocol) Either orally over days 8–11 or intravenously on day 8 of the 21-day protocol (+ furosemide 2.2 mg/kg <sup>1</sup> intravenously bolus) Once daily taper and discontinue over 24–32 weeks
Vinblastine/Lomustine (CCNU)/Prednisone	VBL: 2 mg/m <sup>2</sup> intravenously CCNU: 70 mg/m <sup>2</sup> orally Prednisone: 0.5 mg/kg <sup>1</sup> orally	Once: week 1 Once: week 3; repeat VBL/CCNU every 2 weeks for 6 months Every other day
Lomustine <sup>217</sup>	60–90 mg/m <sup>2</sup> orally	Once every 3 weeks, four times

### External beam radiation therapy

The use of external beam radiation therapy is recommended for postoperative residuals, microscopic or subclinical disease, if further resection is not possible. Furthermore, radiation therapy can also be applied preoperatively in order to reduce tumour size prior to surgery.<sup>211</sup> Radiation therapy at low doses of 40–45 Gy or hypofractionated protocols yield a 1-year tumour control rate of about 44% to 78% for macroscopic tumours.<sup>109,121,212</sup> Control rates and duration may be improved with the application of higher total doses of up to 57 Gy, but the use of radiation as a single modality for MCT should be reserved for nonresectable locations.<sup>211</sup> Combined treatment of radiation therapy and surgery greatly improves tumour control, especially in cases where surgery alone is incapable of controlling the disease due to functional or cosmetic constraints.<sup>211</sup> DFIs of 94% at 1 year and 80% at 5 years have been reported following about 54 Gy radiation of intermediate-grade, stage 0 disease.<sup>203,204</sup> As previously mentioned, prophylactic irradiation of the regional lymph node underlies debate, but some institutions routinely treat negative lymph nodes with 48 Gy (16 fractions) and positive nodes with 54 Gy (19 fractions).<sup>196,204</sup> For loco-regional disease with systemic spread, palliative radiation can be considered as an (additional) option for local palliation of disease.

Generally, a minimum of a 3 cm margin of normal appearing skin is included in the radiation field. For lymph nodes, the prescribed radiation fields are generally succinct to include the node and about 1 cm margins. For definitive therapy, total doses of 48–57 Gy are described in 15–19 fractions.<sup>211</sup>

### Chemotherapy

The use of chemotherapy in the management of canine cutaneous MCTs primarily aims at the treatment of disseminated, nonresectable or high-grade tumours and may be considered for microscopic disease, if radiation therapy, the primary choice for microscopic residual disease, is not feasible. Various chemotherapeutic drugs and protocols have been evaluated in macroscopic canine MCTs<sup>209,213–218</sup> and several studies have used chemotherapy as adjuvant after surgical removal and/or irradiation of the tumour.<sup>112,209,213,218–220</sup> The response of cutaneous MCTs to prednisone as a single agent,<sup>221</sup> prior to surgical excision

or irradiation (neoadjuvant),<sup>121,202</sup> and in combination with chemotherapeutic agents such as vinblastine,<sup>112,218–220</sup> vinblastine/cyclophosphamide<sup>209,213</sup> or cyclophosphamide/vincristine/hydroxyurea<sup>214</sup> was described in several reports.

The response rate to single agent chemotherapy for measurable disease was reported in four peer-reviewed publications and ranges from 7% to 13% for the vinca alkaloids (vincristine: 7%, vinblastine: 11.8% and vinorelbine: 13%) to 44% for the alkylating agent CCNU.<sup>215–217,222</sup> Only the duration of response to CCNU monotherapy was described and found to be short-lived with 2.6 months duration.<sup>217</sup> While the overall objective response to single agent systemic prednisone therapy has been observed to be 20% to 70%, there was a lack of durable efficacy in the only controlled clinical trial.<sup>221</sup> In the other two studies describing the response of cutaneous MCTs to oral prednisone treatment, response was evaluated when the drug was used as neoadjuvant in order to facilitate combined treatment such as surgery or radiation therapy.<sup>121,202</sup> However, the inclusion of prednisone into chemotherapeutic protocols has produced overall response rates of 47% to 64%.<sup>209,214,218</sup> and the 47% overall response found for the combined therapy of vinblastine and prednisone and the 44% response rate to CCNU monotherapy has to date directed these protocols as standard of care in the management of this disease. While a pilot study using a cyclophosphamide/vinblastine/prednisone combination demonstrated a 78% response rate in dogs with measurable metastatic MCT,<sup>213</sup> the efficacy of this protocol combining vinca alkaloid with an alkylating agent and prednisone has been recently confirmed with a measurable 64% response of comparable duration to previously described combined protocols.<sup>209</sup>

For combined therapy aiming at adequate local control of the primary and regional metastatic tumour with surgery and/or radiation therapy, the several chemotherapy protocols have shown to increase the DFI up to 43.5 months (Table 4).<sup>112,209,218,219</sup> Trials including CCNU as part of combined chemotherapeutic protocols for cases with or without adequate local control of the primary tumour are underway with results presented at scientific meetings, but confirmed peer-reviewed results are still pending.

The prevalence of toxicity was described to be up to 26% for combined therapy of vinblastine/prednisone, with



**Table 5.** Medical treatment of systemic symptoms related to mast cell degranulation

In case of ...	Drug group	Example	Dose
Suspected release of vasoactive substances by degranulating MCTs	H <sub>1</sub> blocker	Diphenhydramine	2–4 mg/kg <sup>1</sup> orally twice daily
	H <sub>2</sub> blocker	Ranitidine	2 mg/kg <sup>1</sup> orally twice daily
		Cimetidine	4 mg/kg <sup>1</sup> orally three times daily
Evidence of gastric or duodenal ulceration	Proton pump inhibitor	Omeprazole	0.5–1 mg/kg <sup>1</sup> orally once daily
	Sucralfate		0.5–1 g orally three times daily

Adapted from Thamm & Vail 1999.<sup>112</sup>

5% to 6.5% of moderate to severe toxicities including vomiting and/or neutropenia with fever necessitating hospitalization or discontinuation of treatment.<sup>112,218,219</sup> In the study using vinblastine/cyclophosphamide/prednisolone, the toxicities were described to be mild with 11.4% to 14.2% mild myelosuppressive and 2.8% moderate gastrointestinal toxicity.<sup>209</sup> The acute dose-limiting neutropenia after administration of CCNU was found to be moderate to severe in up to 41%.<sup>217</sup> CCNU has also been associated with cumulative, dose-related chronic hepatotoxicity and other toxicities such as fever, ascites and pleural effusion.<sup>217,223</sup>

### Medical treatment of systemic signs

For clinically symptomatic problems related to excessive histamine release, a combination of H<sub>1</sub> and H<sub>2</sub> blocker therapy is recommended. Recommendations include the use of these agents for systemic or local signs (Darier's sign), cytoreductive surgery (where the tumour is likely to be manipulated) and where degranulation is expected to occur *in situ* (treatment of macroscopic disease with radiation or chemotherapy) (Table 5).<sup>66,121</sup>

### Other treatment modalities

Other local therapies, including intralesional brachytherapy,<sup>223</sup> photodynamic therapy<sup>224,225</sup> and the use of intralesional corticosteroids or deionized water,<sup>129,226–228</sup> have been described, but thorough investigation of these treatments in large patient populations is lacking. In addition, these treatment modalities negate the possibility to assess local tumour control by histological evaluation of margins.

Several drugs targeting the KIT tyrosine kinase receptor are being tested *in vitro*<sup>59</sup> and some clinical response in dogs with MCTs has already been noted in a phase I study of a tyrosine kinase inhibitor.<sup>229</sup>

The use of biological response modifiers (i.e. agents that modify the relationship between the tumour and host) have been studied for years in veterinary medicine. The approach for treatment of cutaneous canine MCT was made by using the immunomodulatory and antitumour effect of LDI-100, a preparation containing human chorionic gonadotropin and bacillus Calmette–Guerin. The 28.6% clinical responses were comparable to single-agent vinblastine chemotherapy, however, with significantly less side effects.<sup>222</sup>

### Conclusions

Dogs have a unique risk to develop cutaneous MCTs, which account for up to 21% of all skin tumours. The aetiology of MCTs is probably multifactorial and, to some degree, influenced by genetic components. Mutations in

the proto-oncogene *c-kit* are present in up to 40% of all dogs with MCTs, indicating a role of stem cell factor receptor in the aetiology of some canine MCTs.

The diagnostic workup of dogs with a suspected MCT includes a definitive presurgical diagnosis with cytology and/or histopathology, clinical staging, the screening for metastasis, as well as documentation of clinical paraneoplastic signs. The diagnosis of MCTs by FNA cytology or histopathology is straightforward in the majority of cases. Accurate prognosis is, however, more challenging. Histological grading is based on the use of subjective parameters and has proved of limited value for predicting biological behaviour of intermediate-grade MCTs. Investigation of objective parameters likely to be useful for prognostication, including mitotic index, various proliferation markers, KIT- and p53 expression, KIT and tryptase staining pattern as well as IMVD have been carried out. Among these, MI, Ki67 and AgNOR expression currently appear to be the most useful parameters, although the studies have used different cut-off values and thus, results are not yet easy to use in a diagnostic setting.

The optimal management of MCTs is still a challenge for oncologists because of their diverse presentation and behaviour. Improved understanding of the natural history and prognostic indicators of MCTs, as well as the role and indications for multimodality therapy has resulted in increasingly better outcomes.

Local disease control remains the overriding concern, as inadequate initial local treatment often leads to recurrences. The timing and integration of various treatment modalities are often specific to the clinical situation, and this coordination is best achieved by preoperative multidisciplinary evaluation of the patient.

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**Résumé** Les mastocytes (MCs) sont connus pour leur potentiel de transformation néoplasique en mastocytomes solitaires ou multiples (MCTs), ainsi qu'en mastocytose viscérale ou systémique. Les chiens présentent un risque unique de développer un mastocytome, qui représente entre 7% et 21% de toutes les tumeurs cutanées canines. L'étiologie des MCTs canines est inconnue mais est probablement multifactorielle. Cet article décrit les connaissances sur la pathogénie, la présentation clinique, les facteurs pronostiques, les clés diagnostiques incluant le stade clinique, les données cytologiques et histologiques et les différents systèmes de grading qui ont été mis au point en fonction de la morphologie, des marqueurs de prolifération et d'autres facteurs comme la densité vasculaire. En outre, des informations détaillées sur les protocoles thérapeutiques sont présentées.

**Resumen** Los mastocitos son bien conocidos por su transformación maligna en mastocitomas cutáneos solitarios o múltiples, así como el desarrollo de mastocitosis sistémica. Los Perros tienen un riesgo único para el desarrollo de mastocitomas cutáneos, en esta especie representan de un 7 a un 21% de todos los tumores de la piel. Se desconoce la etiología de los mastocitomas pero posiblemente es multifactorial. Este artículo hace una revisión del conocimiento actual de la patogénesis, presentación clínica, factores clínicos en el pronóstico, el trabajo diagnóstico incluido el estadio clínico, los hallazgos citológicos, las características histológicas y los diversos sistemas de gradación evaluados en base a la morfología, la valoración de marcadores de proliferación y otros factores como la densidad vascular. Además se incluye información detallada acerca de los protocolos actuales en el tratamiento de mastocitomas caninos.

**Zusammenfassung** Mastzellen (MCs) sind für ihre neoplastische Transformation zu einzelnen und multiplen kutanen Mastzelltumoren (MCTs), sowie für die viszerale und systemische Mastozytose gut bekannt. Hunde tragen ein besonderes Risiko kutane MCTs zu entwickeln, die 7 bis 21% aller caninen Hauttumoren ausmachen. Die Ätiologie der caninen MCTs ist unbekannt, aber möglicherweise multifaktoriell. Dieser Artikel gibt eine Übersicht über das momentane Wissen in Bezug auf die Pathogenese, die klinische Präsentation, die klinischen prognostischen Faktoren und die diagnostische Aufarbeitung. Letztere beinhaltet das klinische Staging, die zytologischen und histologischen Befunde und die verschiedenen Beurteilungssysteme, die anhand der Morphologie, durch die Beurteilung von Proliferationsmarkern und anderen Faktoren, wie die Gefäßdichte, evaluiert wurden. Weiterhin werden detaillierte Informationen über derzeitige Behandlungsprotokolle für canine MCTs gegeben.