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Overview on Mast Cell Tumors in Canines

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Abstract

Among adult dogs, cancer is the most common cause of death, between these mast cell tumors (MCTs) account for 7-21% of all skin neoplasias.

The most common form of MCTs are the cutaneous MCTs (85%) followed by subcutaneous MCTs (15%), extracutaneous MCTs are rarely occur.

The clinical appearance and signs of MCTs are variable, therefore the suggested method for diagnosis is the fine needle aspiration (FNA), as it can be performed in conscious animals.

Grading of the MCTs are important as it shows correlation with the prognosis and it modifies the treatment: there are 2 grading systems in use. While Patnaik's system is having 3 grades Kiupel only established 2: low or high grade tumors. Full staging is only recommended in high grade MCTs, when additional therapy is taken into consideration.

For 90% of low-grade MCTs, surgery completely cures the disease, adjunctive therapy is always suggested for high grade MCTs. This usually being made with irradiation, chemotherapy and tyrosine-kinase inhibitors. In some cases, especially when excision is not possible, these therapies can be used as primary treatment options. Neoadjuvant chemotherapy can be used in some cases for downstaging, to increase the chances of full excision and thus improve the prognosis.

Table of Contents

List of abbreviations

5-HT	5-Hydroxytryptamin (serotonin)
ALT	Alanine-aminotransferase
CCNU	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (Lomustine)
DFI	Disease-free interval
FNA	Fine needle aspiration
Gy	Gray
H2-Receptor	Histamine H2 receptor
IFN-γ	Interferone gamma
IL	Interleukin
ITD	Internal tandem duplication
MCM7	Minichromosome Maintenance Complex Component 7
MCT	Mast cell tumor
MCTs	Mast cell tumors
MI	Mitotic index
NOR	Nucleolar organizer region
PCNA	Proliferative cell nuclear antigen
PCR	Polymerase chain reaction
PDGFR	Platelet derived growth factor receptor
PDGFR-β	Platelet derived growth factor beta
SCF	Stem cell factor
SCFR	Stem cell factor receptor
TT	Tigilanol tiglate
VEGFR-2	Vascular endothelial growth factor receptor 2
WHO	World Health Organization

1. Introduction

Friedrich von Recklinghausen made the first description of mast cells in 1863 and labelled them granular cells. Wilhelm Waldeyer found plasma cells in a tissue sample, which later Paul Ehrlich (1877) named “Mastzellen”, the German term for mast cells. It translates to „well-fed cell“ which Ehrlich found fitting because of the extensive number of granules (Zhang & Shi, 2012). In 1869, Nettleship and Tay gave the first description of mastocytosis which up until then was just believed to be a rarely occurring form of urticaria. The term mastocytosis describes a group of disorders characterized by abnormal growth and accumulation of mast cells in one or more organ systems (Valent et al., 2001).

It took 64 years from the description of mast cells until the first publication of mast cell tumors as a specific neoplastic condition. The pathologist F. Bloom (1942) established the term mastocytoma and described the special features of treatment (Saunders, 1985). Mastocytoma refers to the neoplastic proliferation of mast cells, in other literature it might also be found as mast cell tumors or mast cell sarcomas.

Mast cell tumors (MCTs) are frequently seen in the everyday work of small animal practitioners. Having well-founded and up-to-date knowledge about the current advances in the diagnosis and treatment of MCTs helps providing an optimal result to the client and quality treatment for the patient. Therefore, hereinafter I want to summarize the current advances on mast cell tumors and give an overview about the up-to-date treatment protocols and prognostic factors of mast cell tumors.

2. Epidemiology

In the recent years there have been huge improvements in the conditions for pet animals concerning the keeping, nutrition, vaccination, better preventive and therapeutic medical practices and generally a deeper connection between owner and pet. These results in increased age of dogs and consequently an increased likelihood to develop cancer (Tanaka et al., 2020).

The study made by Adams in 2010 examined the death of 15.881 dogs in the UK, in 27% of the cases cancer was the reason of death.

In a study from 2011, Fleming et al. classified 6.855 juvenile (up to 1 year) and 41.259 adult (1 year or greater) dogs for the proportions of death attributable for pathologic processes (see **Figure 1**). Their study shows the importance of oncologic research in veterinary medicine as in adult animals the percentage of neoplastic processes accounts for approximately one third of all deaths of dogs.

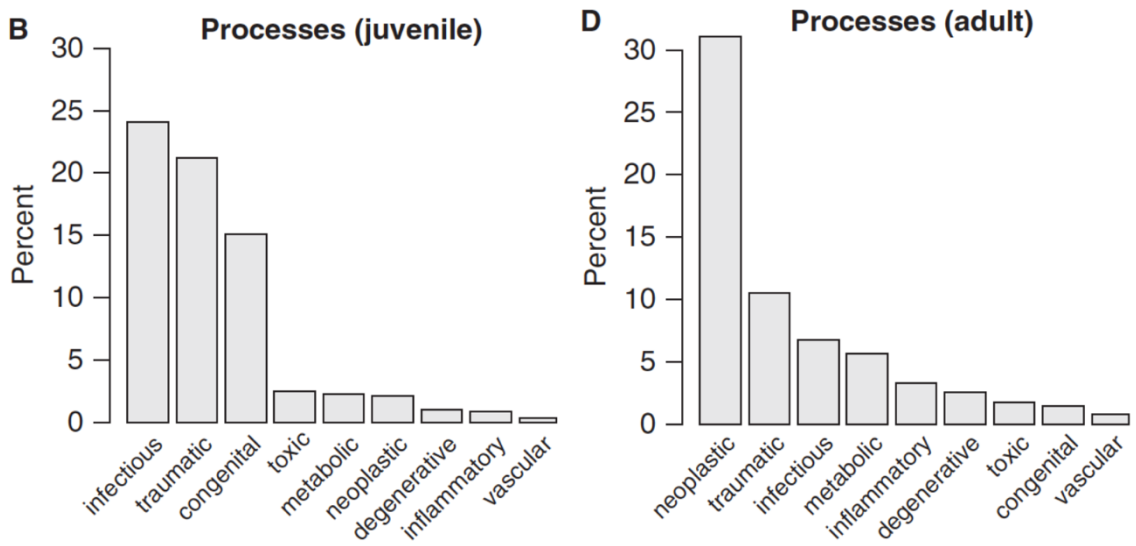

Figure 1: Proportions of death attributable for pathologic processes (Fleming et al., 2011)

Bostock (1986) examined 5.000 skin biopsy samples approximately 50% were well-differentiated neoplasms with good prognosis, 30% were potentially malignant neoplasms and 20% were non-neoplastic inflammatory or degenerative lesions.

Dobson et al. (2002) made research on 2.546 cases of neoplasia in the United Kingdom and found skin and soft tissue the most common tumor site with a 7 times higher incidence compared to the second most common site of the study (see **Figure 2**).

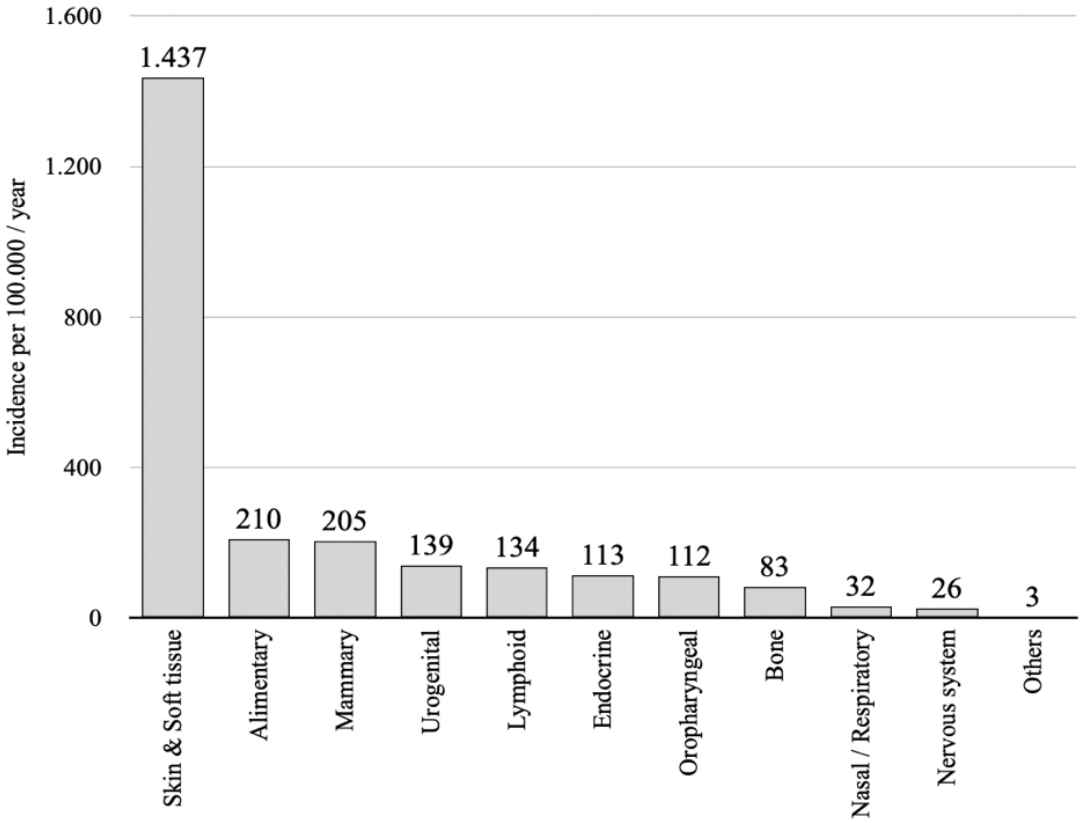

Figure 2: Incidences of different tumor sites (Dobson et al., 2002)

Villamil et al. (2011) analyzed the frequency of skin neoplasia and reported a change from 1,9% (1964) to 3,6% (2002). They suggest that this finding could be based on the improving diagnostic possibilities, especially the increased use of immunohistochemical staining rather than an increase incidence. Grüntzig et al. (2016) concluded that the relative frequency of mast cell tumors in the time frame of his retrospective study (1955 to 2008) rose from 2,1% to 8,4% of the overall tumor diagnoses. Furthermore, Dobson et al. (2002) reported the most common single tumor types and mast cell tumors came out to be the fifth most common neoplasia in dogs with an incidence of 129 per 100.000 per year (see **Figure 3**). It should be mentioned that the population used in this study exclusively consists of insured dogs. Thereof the population is likely to show an increased percentage of pure bred and young dogs compared to the general population in the UK.

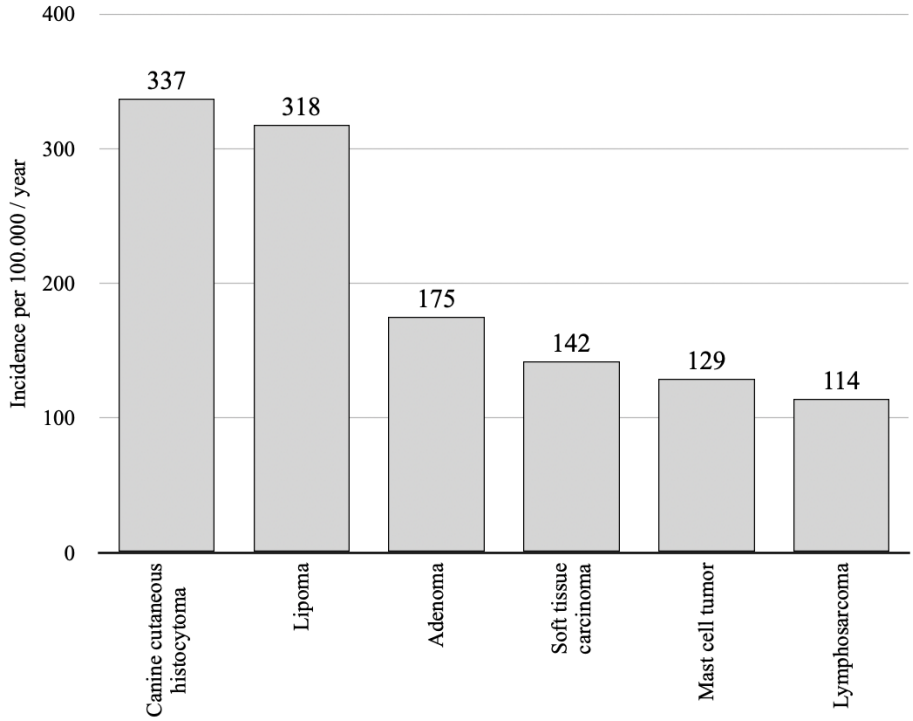

Figure 3: Incidences of the most common single neoplasms (Dobson et al., 2002)

Another study was done by Graf et al. in 2018 examining 11.740 skin tumors in Switzerland and an incidence of 60,4 per 100.000 per year was calculated for the development of mast cell tumors. Shoop et al. (2015) detected a prevalence of 0,27% (453 out of 168.636) for mast cell tumors in an English canine population.

Different studies as seen in **Table 1** suggest that mast cell tumors account for somewhere between 5 to 12% of all reported tumors. This shows us the importance of studies and knowledge about this topic. Among skin tumors, MCTs account for 7–21% of skin tumors (Kiupel, 2017) and thus are an important differential diagnosis among skin lumps.

Table 1: Percentage of mast cell tumors among canine tumors

Author	Year of Publication	Timeframe	Country	MCT (Total cases)	Percentage
Cohen et al.	1974	1952 - 1964	Pennsylvania, USA	226 (2.550)	8,86 %
Bastianello	1983	1935 - 1974	South Africa	432 (3.388)	12,7 %
Grabarević et al.	2009	2002 - 2006	Croatia	106 (1630)	6,5 %
Šoštarić-Zuckermann et al.	2013	2006 - 2009	Croatia	77 (1568)	4,91 %
Grüntzig et al.	2016	1955 - 2008	Switzerland	4.415 (63.214)	6,5 %
Baioni et al.	2017	2001 - 2008	Italy	73 (1.175)	6,21 %

2.1.BREED

Several breeds have been identified to be predisposed for the development of mast cell tumors. Shoop et al. (2015) discovered that there is a significantly larger proportion of specific breed types (purebreds) compared to crossbreeds for the development of mast cell tumors. The breeds with a higher incidence are the Boxer, Labrador & Golden Retriever, Bull Terrier, Boston Terrier, Shar Pei, Pug, Weimaraner, Staffordshire Bullterrier, Parson Jack Russel Terrier, American Staffordshire Terrier, French Bulldog and Dachshund (Head, 1958; Hottendorf & Nielsen 1967; Peters, 1969; Miller, 1995; McNiel, 2006; Warland & Dobson, 2013; Shoop et al., 2015; Grüntzing et al., 2016; Śmiech et al., 2019).
This predisposition does not necessarily only affect the occurrence of MCT, Boxers for example have shown to be generally predisposed for tumor development (Baioni et al., 2017). Śmiech et al. (2019) assume a genetic background for the development of mast cell tumors based on the high occurrence of certain breeds in their study. A pedigree analysis would be required to solidify this presumption to exclude possible inherited factors.
Interestingly, according to Śmiech et al. (2019) some breeds are not just predisposed for the development of MCTs but also for the occurrence of specific grades of MCTs. Boxers, Labrador Retrievers, French Bulldogs, Golden Retrievers and American Staffordshire Terriers were mainly affected by low-grade MCTs while Shar Peis and American Staffordshire Terriers have the highest risk of developing high-grade MCTs (Kiupel grading system). Golden Retriever are predisposed for multiple MCT (Murphy, 2006), as well as Pugs (McNiel, 2006).

2.2. AGE

Hottendorf & Nielsen (1967) described the mean age for the occurrence of mast cell tumors to be 8,2 years, the median was found to be 8,6 years. Strefezzi et al. (2003) found the mean age to be 8,5 years. It should be noted that with old age the risk of tumor development increases. A comparison of the onset of MCTs from two studies can be seen in **Figure 4**.

Śmiech et al. (2019) figured out that the highest risk of development of MCT in Labrador Retrievers is between the age of 4–6 years while for Boxers and French Bulldogs it is between 7–10 years. Mochizuki et al. (2016) showed that breeds of small and medium size had the tendency to develop MCTs at an older age while breeds of the bulldog origin (Boxer, French Bulldog, Bulldog, American Staffordshire Terrier, Staffordshire Bull Terrier and Boston Terrier) showed the tendency to develop MCTs at younger age. It should be taken into consideration that small breeds are living longer in average.

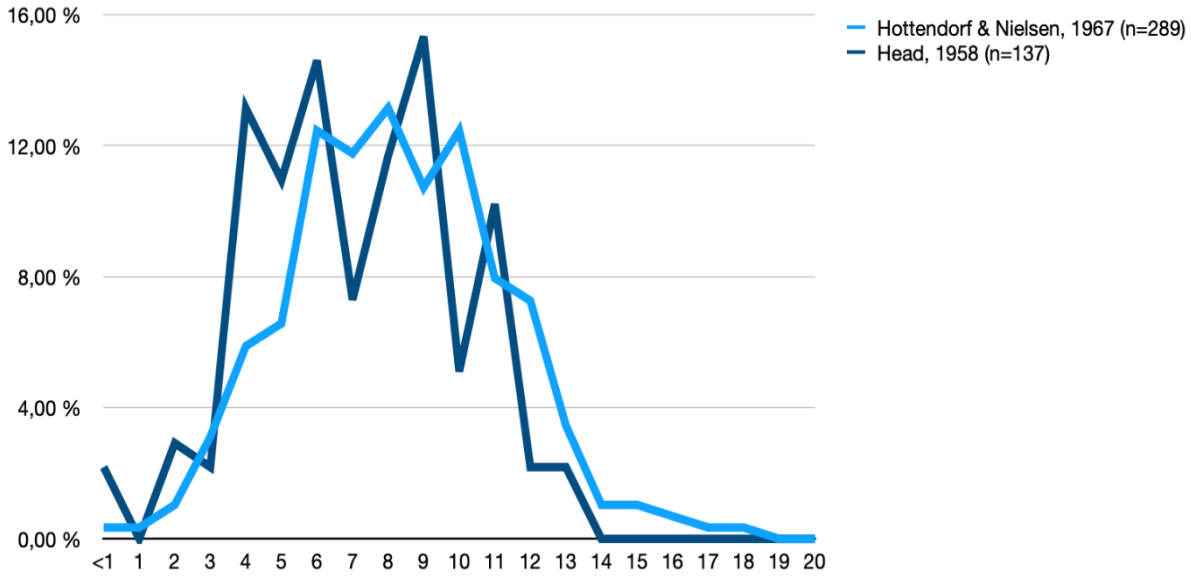

Figure 4: Comparison of age of occurrence of mast cell tumors

2.3. GENDER

Hottendorf and Nielsen (1967) showed that from 272 dogs examined, 51,8% (n=141) were female. Assuming an equal sex distribution, no predilection for gender could be shown. This agrees with the finding of other studies (Frese, 1968; Shoop et. al., 2015; Śmiech, 2019). Neutered male and female dogs show an increased incidence for mast cell tumors as well as skin tumors in general (Grüntzig, 2016).

3. Physiology and Histology of mast cells

3.1. LIFE CYCLE

Mast cells originate from the hematopoietic tissues and their progenitor cells are released into the peripheral blood stream and migrate to connective or mucosal tissues. There, they proliferate and differentiate into morphologically identifiable, mature mast cells (Kitamura, 2007). The migration to peripheral tissues is controlled by chemotactic agents and growth factors. While connective tissue mast cells have an average life span of less than 40 days, mucosal mast cells life span is more than 6 months (Tizard, 2004). Watt & Ennis (2004) found that mast cells can regulate their own numbers and phenotypes in tissues through the action of cytokines.

3.2. MORPHOLOGY

Mast cells are mononuclear round cells of round to polygonal shape with a diameter ranging from 9–20µm. Their nucleus is centrally located and appears as large and bean shaped, although usually it cannot be identified due to the high number of metachromatic granules in the cytoplasm. The cytoplasm of mast cells appears pale pink. Mast cells can be distinguished into two major types based on their location. While mast cells of the connective tissue are 9 to 10 µm in diameter and in their cytoplasm only a few, variable-sized granules occur, mast cells of the mucosal tissues are 19–20µm in diameter filled with many, uniform granules (Kiupel, 2017; Tizard, 2004).

3.3. FUNCTIONS

Mast cells play an important role in inflammation as they mediate vascular permeability, vasodilation, anticoagulation and activation of eosinophil and neutrophil granulocytes (London & Seguin, 2003). On their surface, mast cells express a huge variety of receptors for binding and detecting antigens and consequently releasing their chemical mediators. These mediators can be summed up into three main groups:

1. **Granule content** (histamine, heparin, chondroitin sulfate, mast cell proteases)
2. **Lipid mediators** (prostaglandins, leukotrienes, and platelet-activating factor)
3. **Cytokines** (tumor necrosis factor-a, IL-3, IL-4, IL-5, and IL-6 (interleukin)

Theoharides et al. (2007) could show that a differential release of mediators from mast cells is possible. Selected mediators can be released from mast cells without full degranulation and only some molecules are essential for the degranulation process.

4. Pathogenesis

Neoplasms are growths of cells, that are derived from the normal tissue but have undergone genetic changes, making them unresponsive to normal growth controls. Consequently, the tissues are expanding beyond their normal anatomic boundaries.

Benign tumors are not invading surrounding tissues or spread to new locations within the body. In general, benign tumors are curable and rarely result in death. Malignant tumors on the other hand can invade surrounding tissues and form metastases throughout the body and, untreated, sooner or later resulting in the death of the host (McGavin & Zachary, 2007).

Mast cell tumors belong to the group of mesenchymal tumors and can occur in a benign and malignant form. An estimate of 50% of MCTs are malignant (Ma et al., 1999).

The exact aetiology of mast cell tumors is still unknown, a multifactorial background like in most neoplasms is likely though (Welle, 2008). Several studies reviewing the breed distribution of MCTs suggest a genetic component (Śmiech et al., 2019; Peters, 1969).

Webster et al. (2007) suggest a role of c-Kit mutations in the progression of canine MCT as aberrant KIT protein localization and internal tandem duplication are associated with increased cellular proliferation. Meyer et al. (2012) suggest the involvement of CD25 in early MCT development and further the involvement as a stimulatory factor in grade 1 MCTs.

4.1. STEM CELL FACTOR RECEPTOR (SCFR)

SCFR (also called KIT) is a tyrosine kinase receptor encoded by the proto-oncogen c-Kit. The SCFR is activated by binding of stem cell factor (SCF) (Lennartsson & Rönnstrand, 2012). In physiologic mast cells, the interaction of SCF with SCFR is critical for cell differentiation, maturation, proliferation, cell survival and function (Downing, 2002).

London et al. (1996) were able to show SCFR on canine neoplastic mast cells and suggested an involvement in the aetiology of mast cell tumorigenesis.

Mutations of c-Kit were found in 15% to 40% of all cases of canine cutaneous MCTs (Welle et al., 2008). This indicates that c-Kit mutations are playing an important role in the aetiology of MCTs, but mutations of other genes or other etiological factors are likely to exist. SCF-independent SCFR activation was shown in cells with c-Kit mutations. The exact mechanism was not able to be identified yet, it is believed to be due to the relief of auto-inhibitory mechanisms though (Lennartsson & Rönnstrand, 2012). Takeuchi et al.

(2013) were able to show that mutations of the KIT receptor can lead to its continuous activation.

Mutation of c-Kit is a gain of function mutation and several different mutations of c-Kit have been identified. Mainly, mutations were found on exon 11, but mutations on exon 8 and 9 were also identified (Letard et al., 2008).

4.2.PARANEOPLASTIC SYNDROME

Paraneoplastic syndrome is the dysfunction of an organ or tissue due to a neoplasia but is not directly related to the invasion of the affected organ or tissue by the primary tumor or metastasis (Bateman, 2003). In MCTs, paraneoplastic signs are related to the release of mast cell granules and the actions of the constituents, such as histamine, heparin or proteases (London, 2003). Oedema, swelling and ulceration can be observed at the primary tumor site. Furthermore, delayed wound healing and coagulation abnormalities may evolve (Blackwood et al., 2012; Welle et al., 2008)

Gastrointestinal symptoms are the most common systemic signs (Blackwood et al., 2012). Histamine stimulates the gastric H2-receptors (histamine H2 receptor), resulting in over-secretion of hydrochloric acid and gastric hyper-motility (Howard, 1969). Increased levels of hydrochloric acid in the stomach result in ulceration, the clinical symptoms that may be seen are anorexia, vomiting, melena, haematochezia and abdominal pain (Welle et al., 2008; O'Keefe, 1990).

In their study from 1969, Howard et al. demonstrated gastrointestinal ulcerations in 83% of the cases with MCT (20/24). In a population of 17 dogs with MCT, Fox et al. (1990) identified 35% to have clinical symptoms of gastrointestinal ulceration, yet absence of clinical signs does not mean absence of gastrointestinal ulceration. Secondary, iron deficiency anaemia and peritonitis due to gastrointestinal perforation can evolve.

Rarely, anaphylactic reaction can occur due to massive release of histamine (Blackwood et al., 2012; Welle et al., 2008).

5. Diagnosis

In recent years, the importance of differentiation between cutaneous and subcutaneous MCTs was figured out as they carry different sets of prognostic values (Thompsen et al., 2011) and thus, in this chapter they will be presented separately.

Welle et al. (2008) describe 3 goals of MCT diagnosis:

1. Definite diagnosis by cytology and/or histopathology
2. Clinical staging
3. Documentation of paraneoplastic clinical signs

5.1. CUTANEOUS MAST CELL TUMORS

Cutaneous MCTs are the most common form of MCTs (Kok et al., 2019). Kok et al. (2019) distinguished cutaneous MCTs from subcutaneous MCTs and found cutaneous MCTs accounting for 85% and subcutaneous MCTs for 15% of all skin MCTs.

5.1.1. CLINICAL APPEARANCE

The clinical appearance of cutaneous MCTs is highly variable and a relation to the tumor grade has been suggested. The size of MCTs can vary between a few millimeters and large-sized masses. Consistency can vary from soft, lipoma-like to firm, nodular (London & Seguin, 2003; Kiupel, 2017).

MCTs have been described as nodular rashes, diffuse swellings or erythematous tumors. Commonly, MCTs are hairless, solitary lesions (Kiupel, 2017; Blackwood et al., 2012) although in 9–21% of cases, multiple MCTs are present (O'Connell & Thomson, 2013; Mullins et al., 2006; Hottendorf & Nielsen, 1967).

In most cases, well-differentiated MCTs are slow growing, hairless, solitary lesions, while poorly differentiated MCTs show rapid growth and present as pruritic and ulcerated lesions with sometimes further lesions, so called satellite lesions, close by (Blackwood, 2012).

Although mast cell tumors that grossly appear as aggressive (large, invasive, severely ulcerated) show a tendency to be malignant (Kiupel, 2017), canine MCTs stay unpredictable and should always assumed to be malignant until proven otherwise (Ginn et al., 2000). Hence, diagnosis of MCT should never be based on the tumor appearance (London & Seguin, 2003).

Data regarding the site distribution of cutaneous MCTs from 3 studies is shown in **Figure 5**.

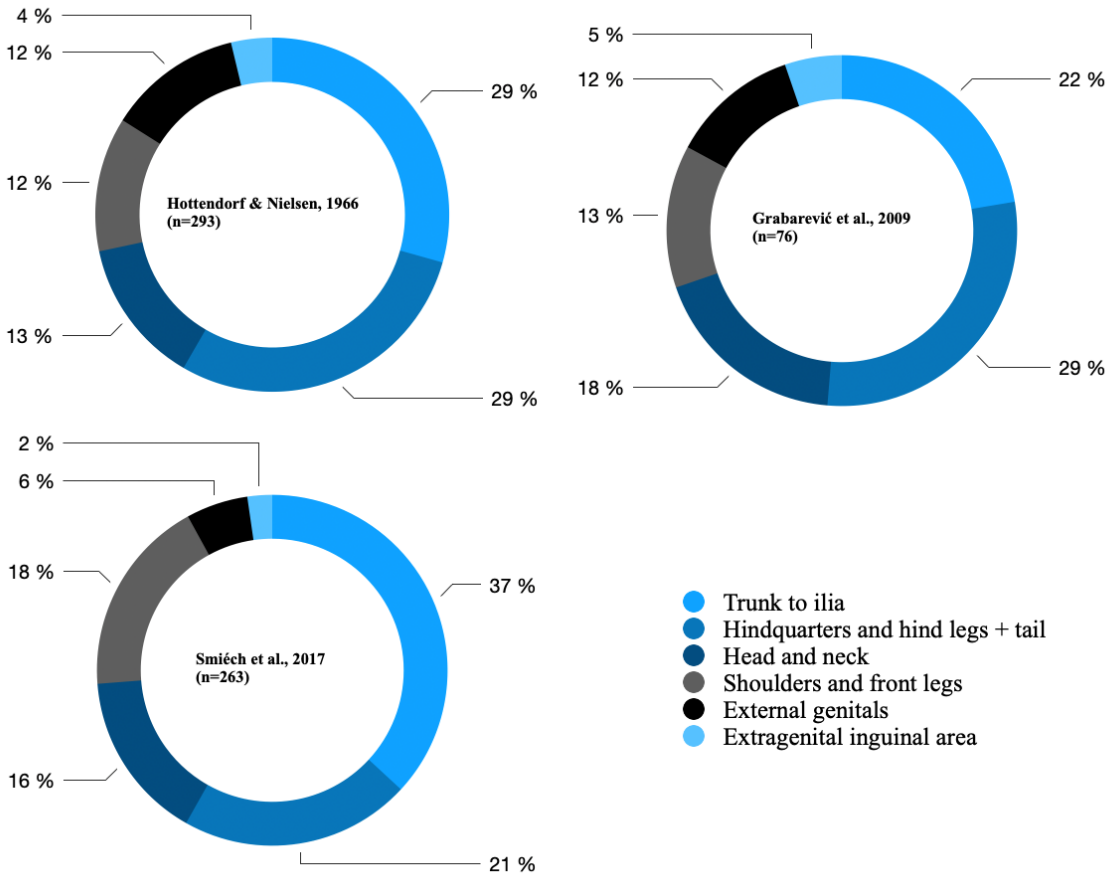

Figure 5: Occurrence of mast cell tumors in different body regions (Hottendorf & Nielsen, 1966; Grabarević et al., 2009; Smiéch et al., 2017)

Previously, the location of cutaneous MCTs was believed to be directly related to the prognosis. Blackwood et al. (2012) noted that this believe may account to the decreased possibility of adequate local surgical control rather than being an actual prognostic factor. O'Connell et al. (2013) found no location to be associated with a worse prognosis and the time to disease progression or death in MCTs located on the head, trunk or in mucocutaneous junctions were similar to that of MCTs in other locations. Studies examining the prognosis of inguinal and perineal MCTs and found no difference in survival time or tumor-free interval compared to MCTs in other locations (Cahalane et al., 2004; Sfiligoi et al., 2005).

Whether the occurrence of multiple cutaneous MCTs is a negative prognostic factor or not is controversially debated. Kiupel et al. (2005) found it to be a negative prognostic factor, while O'Connell et al. (2013) found no influence on the prognosis and proposed to

evaluate each MCT as an individual tumor. Mullins et al. (2006) found multiple MCTs to be associated with a low rate of metastasis and a good prognosis for long term survival after complete excision of all MCTs.

Hahn et al. (2004) found the size of MCTs to be a prognostic factor as MCTs smaller than 3 cm were found to have a longer remission time (31 months) than MCTs larger than 3 cm (24 months).

5.1.2. CLINICAL SIGNS

Locally, swelling, erythema, pruritus and pain were reported in dogs with MCT (Mullins et al., 2006). Upon manipulation, neoplastic mast cells degranulate and release histamine, as well as other vasoactive mediators, into the surrounding tissue. This results in the formation of Darier's sign, presenting as oedema, erythema and wheal formation (Kiupel, 2017; Welle et al., 2008). Fröberg et al. (2009) suggest that serotonin (5-HT) released from neoplastic mast cells could also produce the Darier's sign, as it has been shown to be able to produce itching, flare and wheals when applied to human skin. Further, this suggests the use a 5-HT antagonist along with antihistamines to reduce symptoms.

In 1987, O'Keefe et al. examined 16 dogs with MCT and found 50% of them with systemic signs related to the MCT. Most prevalent were gastrointestinal signs due to the ulcerogenic characteristics of MCTs. Gastrointestinal ulceration should be suggested in cases of anorexia, vomiting, melena, hematochezia or abdominal pain (Welle et al., 2008; O'Keefe, 1990). In severe cases, peritonitis due to gastrointestinal perforation and iron deficiency anaemia due to chronic blood loss could occur (Blackwood et al., 2012; Welle et al., 2008).

Accompanying lymphadenopathy or organomegaly raises the suspicion of metastases (Kiupel, 2017).

5.1.3. CYTOLOGY

Taking into account the variable appearances of mast cell tumors, every mass detected in the skin or subcutaneous tissue has potential to be an MCT. Due to the increase of aggressiveness in surgical therapy required in MCTs compared to other skin masses, diagnosis of MCTs is indispensable before surgery. The easiest and cheapest method for diagnosis of MCTs is fine needle aspiration (FNA), as it can be performed in conscious animals (Blackwood et al., 2012). Griffiths et al. (1984) found FNA to be accurate in diagnosis of MCTs in all cases (37/37), Ghisleni et al. (2006) (25/25) and Simeonov (2010) (29/29) came to similar results.

Krick et al. (2009) reviewed lymph node aspirates of 152 dogs in a retrospective study. A correlation between tumor grade and cytological evaluation of the lymph node was detected and it was concluded that FNA provides a practical and non-invasive tool for staging. Chaffin and Thrall (2001) found lymph node enlargement in only 26% of cases with lymph node metastasis, suggesting a cytologic examination is indicated in all cases of MCT, in which the regional lymph node is accessible.

Alternatively, incisional biopsies can be used for establishing the diagnosis of MCTs, although they require anesthesia and thus are associated with higher costs. Furthermore, wound breakdown is a possible complication (Blackwood et al., 2012). The European Society of Veterinary Oncologists and the Veterinary Cancer Society (2020) suggest cytology over incisional biopsies because incisional biopsy has a risk of underestimating the tumor grade, especially in grade II (Patnaik) or high grade (Kiupel).

Duncan and Prasse (1979) reported differences in the size of mast cells between well differentiated and anaplastic MCTs in a study with 25 MCTs. While in well-differentiated MCTs mast cells were of physiological size (10-20µm), in anaplastic MCTs mast cells ranged between 12–35µm.

Camus et al. (2016) proposed a cytological grading scheme based on the morphologic characteristics of neoplastic cells. Tumors were declared high grade if they were either poorly granulated or showed at least 2 out of 4 of the following features: presence of any mitotic figures, anisokaryosis >50%, binucleation or multinucleation, or nuclear pleomorphism. 152 MCTs were examined with cytological grading and the results were compared to Kiupel's two-tier histological grading system, 88% sensitivity and 94% specificity were found. Thus, cytological grading was shown to be a useful prognostic and therapeutic indicator.

A significant difference in mean nuclear area and mean nuclear perimeter between the nuclei of grade III MCTs and grade I and II MCTs was found in two studies, suggesting the use in cytological differentiation of grade III MCTs from grade I and II (Streffezzi et al., 2003; Maiolino et al., 2005).

It is suggested to always examine the tributary lymph node and in case of enlargement to further examine it with FNA (Kiupel, 2017). It is important to note that mast cells physiologically occur in lymph nodes and results can be misleading (London & Seguin, 2003). Highly indicative of metastatic spread is the presence of clusters or sheets of mast cells, increased mast cell density and a large number of poorly differentiated mast cells in the lymph node (Krick et al., 2009).

In case of suspected or confirmed nodal metastases, full staging including thoracic radiographs, abdominal ultrasound and aspiration of spleen, liver and bone marrow is required (Kiupel, 2017). Therefore, Kiupel (2017) suggests to always perform an abdominal ultrasound in dogs with MCT. Contrary, Pecceu et al. (2019) and Book et al. (2011) have shown that ultrasound alone is poorly indicative for metastases. Hepatic and splenic cytology is advised as a routine procedure in high risk MCTs and should be done regardless of the organ appearance or regional lymph node status (Pecceu et al., 2019; Book et al., 2011). Stefanello et al. (2009) suggest the use of FNA in all cases of cutaneous MCTs.

It must be noted that the physiological occurrence of mast cells in visceral organs and furthermore, other, non-neoplastic conditions with organ mast cell infiltration can be misleading (Pecceu et al., 2019). Finora et al. (2006) concluded that the finding of mast cells in liver and spleen aspirates alone is not a reliable sign for malignancy and further declared routine aspiration of liver and spleen as not useful.

Bone marrow aspirations rarely show positive findings in case of MCTs. Endicott et al. (2007) suggest performing bone marrow aspiration in case of abnormal hemogram (neutrophilia, monocytosis, eosinophilia, basophilia, anemia and thrombocytopenia) or in cases of reoccurring or progressing MCTs.

5.1.4. HISTOPATHOLOGY

Histologic examination is performed with samples obtained from either incisional or excisional biopsy (Blackwood, 2012). As previously mentioned, incisional biopsy comes along with an increased cost and the risk of wound problems (Blackwood, 2012). As only a sample is removed from the tumor it requires an additional surgery after histological examination. Excisional biopsy follows cytological identification of the tumor and is the complete removal of the tumor. Based on the obtained histological sample, margin evaluation and grading should be done to gain further therapeutic and prognostic insights.

Sometimes, neoplastic mast cells show special formations in the tissue sample in form of ribbons or rows. High eosinophil granulocyte counts in the tissues are indicative for MCTs. Some MCTs show signs of oedema and hemorrhages in tissue samples. Further tissue changes possibly to occur are collagenolysis, sclerosis, necrosis and secondary lymphocytic proliferation. Depending on the extent, these changes can mask neoplastic mast cells and complicate the evaluation of margins (Kiupel, 2017).

In many cutaneous MCTs, a reactive halo composed of oedema fluid, inflammatory cells, mast cells and reactive stromal cells surrounding newly formed capillaries complicates margin evaluation. This holo can be up to several centimetres and holds single mast cells as well as satellites (clusters of five or more mast cells). Mast cells in the holo can be neoplastic or attracted by chemokines (Kiupel, 2017).

5.1.5. MARGIN EVALUATION

Although MCTs do not have a capsule, margins in well-differentiated MCTs can be readily identified. Less differentiated MCTs show infiltration into close-by tissues and margin evaluation becomes challenging (Kiupel, 2007). Physiologically occurring mast cells in the surrounding tissues of the MCTs complicate the identification of margins as the differentiation of neoplastic mast cells from normal mast cells is not possible. Furthermore, neoplastic mast cells, contrary to normal mast cells, can secrete cytokines without adequate stimulation and attract further mast cells (Kiupel, 2017).

Kiupel (2017) described a method for the consistent evaluation of margins. A combination of complete tangential margins for “cleanliness” and assessing distance of neoplastic cells to these margins based on radial sectioning of the MCT should be used.

Margins should be reported as the following:

M1 = Margin infiltrated

M2 = Margin is close, within 1-2mm

M3 = margin is clean 2-5mm

M4 = margin is clean >5mm.

5.1.6. GRADING

Grading of MCTs gives important information regarding therapy and prognosis. It is based on histopathology, no reliable grading system based on cytology could be established yet (Kiupel, 2017).

Two different systems are currently in use for the grading of MCTs. Patnaik established a system to divide MCTs into grade I, II and III based on cellular characteristics and tissue infiltration (Patnaik, 1984). The histopathologic characteristics of each individual grade can be seen in **Table 2**.

Grade 1: well differentiated tumors

Grade 2: intermediately differentiated tumors

Grade 3: poorly differentiated tumors

Table 2: Patnaik grading scheme for mast cell tumors (Grabarević et al., 2009)

Histopathologic feature	Grade I	Grade II	Grade III
Cellularity	Low	Medium	High
Cell size	Uniform	Medium anisocytosis	Severe anisocytosis
Giant and binuclear cells	None	Few	Often
Pleomorphism	None	Medium	High
Cytoplasmic granules	Distinct	Visible	Unidentifiable or very low visibility
Nuclei	ovoid, uniform	Anisokariosis	Anisokariosis
Mitosis	None	Medium degree	Numerous
Mitotic index (x40)	<2	2-8	>8

Recently, disadvantages of the Patnaik grading systems were shown in form of high inter-observer differences. Kiupel et al. (2011) had 28 different pathologists evaluate 95 MCTs and for grade III 74,6% of pathologist agreed on the diagnosis, for grade II and I only 63% and 63,1%, respectively, were agreed on. In their study, Northrup et al. (2005) came to similar conclusions examining the differences of 60 MCTs examined by 10 different pathologists. Only in 6,7% (4/60) MCTs, all ten pathologists agreed on the same grade.

Tumor grade is an important prognostic parameter and matching grading results with low inter-observer differences are desirable. In 2011, Kiupel et al. proposed a new grading system consisting of only 2 grades: low-grade and high-grade.

High-grade MCTs are based on the presence of the following criteria:

1. at least 7 mitotic figures in 10 HPF
2. at least 3 multinucleated (3 or more nuclei) cells in 10 HPF
3. at least 3 bizarre nuclei in 10 HPF
4. karyomegaly (nuclear diameters of at least 10% of neoplastic cells vary by at least two times).

In absence of these criteria, a MCT is considered low grade. The evaluation of criteria should be based on vision fields with the highest mitotic activity or with the highest degree of anisokaryosis. The two-tier system provided a 96,8% inter-observer consistency and further, significant association between high grade and mortality, as well as development of additional MCTs or metastases was shown. Furthermore, high-grade tumors were significantly correlated to decreased survival time (Kiupel, 2011).

A promising system for cytological grading was proposed by Camus et al. in 2016. In comparison with histological classification, 88% sensitivity and 94% specificity was found. Hergt et al. (2016) found 86,8% sensitivity and 97,1% specificity.

High grade MCTs were categorised by showing poor granulation or at least two of the following criteria:

Presence of mitotic figures
Presence of binucleated / multinucleated cells
Nuclear pleomorphism
>50% anisokaryosis

Absence of poor granulation or two or more criteria led to the classification as low grade. Even though no full correlation was found between cytologic and histologic grading, cytologic grading can be cheap and helpful tool to plan for excision, especially in regard of margin selection.

5.1.7.STAGING

Full staging is only recommended in high-grade MCTs or when additional therapy is considered, in most cases of low-grade MCTs, the examination of the tributary lymph node is sufficient. Positive findings in the tributary lymph node are suggesting full staging as well. Full staging includes radiographs, abdominal ultrasound and aspirates of spleen, liver and bone marrow (Kiupel, 2017).

According to the WHO (World Health Organization), MCTs can be assigned to four different stages. The characteristics of each individual stage are shown in **Table 3**.

Table 3: Staging of cutaneous mast cell tumors (Owen, 1980)

Stage I Ia. Without systemic signs Ib. with systemic signs	one tumor confined to the dermis no regional lymph node involvement
Stage II Iia. Without systemic signs Iib. With systemic signs	one tumor confined to the dermis regional lymph node involvement
Stage III IIIa. Without systemic signs IIIb. With systemic signs	Multiple dermal tumours or large infiltrating tumor ± regional lymph node involvement
Stage IV	Any tumor with distant metastasis or recurrence with metastasis

Several studies have criticised the staging system, mainly because multiple cutaneous MCTs automatically are assigned to Stage III, even though the occurrence of multiple MCTs alone is not a negativ prognostic factor (O'Connell, 2013; Murphy, 2006). Therefore, based on the assumption that lymph node spread has the worse prognosis compared to multiple cutaneous MCTs, the recommendation to switch criteria between

stage 2 and 3 was made by the European Society of Veterinary Oncologists and the Veterinary Cancer Society (Kiupel, 2017).

A modified staging system added the stage 0, which described a single, incompletely excised tumor without regional lymph node involvement (Hayes et al., 2007).

Hayes et al. (2007) proposed a post-operative staging system, applied to re-evaluate cases after excision of the MCT prior to chemotherapeutic treatment (see **Table 4**).

Table 4: Modified post-operative staging system (Hayes et al., 2007)

Stage -1	Single tumor removed with clean margins Regional lymph node negative
Stage 0	Single tumor incompletely removed Regional lymph node negative
Stage 1	Single tumor completely excised Regional lymph node positive and excised
Stage 2	Single tumor removed with incomplete margins Regional lymph node positive and excised
Stage 3	Single tumor completely excised Regional lymph node positive and remains *in situ*
Stage 4	Single tumor removed with incomplete margins Regional lymph node positive and remains *in situ*
Stage 5	Recurrence or distant metastasis

5.2. SUBCUTANEOUS MAST CELL TUMORS

Subcutaneous MCTs historically were sometimes classified as grade II cutaneous MCTs, even though they don't fit into the grading scheme and different prognostic values are accounting for them (Kiupel, 2017; Thompsen et al., 2011). Compared to grade II cutaneous MCTs, subcutaneous MCTs had extended survival times, DFI (disease free interval), and lower rates of local recurrence or metastases (Thompsen et al., 2011).

5.2.1. CLINICAL APPEARANCE

Subcutaneous MCTs cause bulging of the skin but do not infiltrate the dermis and rarely ulcerate. On palpation, subcutaneous MCTs often appear soft and fleshy, similar to lipomas.

According to my research, no clinical signs were highlighted for subcutaneous MCTs in literature. Based on the similar pathogenesis of cutaneous and subcutaneous MCTs, similar signs can be assumed.

5.2.2. CYTOLOGY

No differences in the neoplastic mast cells of cutaneous and subcutaneous MCTs were identified (Kiupel, 2017), thus cytology cannot differentiate between cutaneous and

subcutaneous MCTs and the differentiation is done by histopathology based on location (Thompsen et al., 2011). As for the diagnosis of subcutaneous MCTs no additional information are available, diagnosis should be done like described for cutaneous MCTs.

5.2.3. HISTOLOGY

Subcutaneous MCTs are in the subcutis and are surrounded by adipose tissue (Kiupel, 2017). They present as demarcated subcutaneous masses with no primary dermal involvement (Newman et al., 2007).

5.2.4. GRADING, STAGING

The subcutaneous MCT was never incorporated into the Patnaik grading scheme, although subcutaneous MCTs possibly were often identified as grade II MCT. To establish a grading system for subcutaneous MCTs will be difficult as the range of alterations is small. (Newman et al., 2007).

No staging system for subcutaneous MCTs is available in the literature. No experience was published for the application of the cutaneous staging system.

5.3.EXTRACUTANEOUS MAST CELL TUMORS

Mast cell tumors have been described in various locations throughout the body. Primary extracutaneous MCTs rarely occur and thus, information about diagnosis, treatment and prognosis is limited. Primary sides for extracutaneous MCTs are gastrointestinal tract, oral cavity, tongue, conjunctiva, salivary gland, nasopharynx, larynx, spinal cord, urethra, liver, spleen, lung (Kiupel, 2017).

Ozaki et al. (2002) described clinical signs due to ulceration in advanced stages of gastrointestinal MCT, also this research group found Maltese dogs to be predisposed for developing gastrointestinal MCTs, accounting for 53,8% (21/39) of the examined cases. Furthermore, miniature breeds accounted for 82% of all cases (32/39).

MCTs of viscera, intestines, bone marrow, nail bed, oral cavity, muzzle or inguinal, preputial, perineal and mucocutaneous areas were correlated with poor prognosis (Welle et al., 2008; Blackwood et al., 2012).

6. Differential Diagnosis

For every skin lump, MCT should be assumed as a differential diagnosis and should only be excluded upon negative cytologic result. Other differential diagnoses are other tumors, non-neoplastic inflammatory changes or degenerative changes. Graf et al. (2018) examined 13.744 skin tumors and showed the distribution per region. **Table 5** lists the likelihood of a skin tumor being a MCT in different regions of the body. Furthermore, the three most common differential diagnoses per region can be seen.

Table 5: Probability of tumors being MCTs in specific body areas (Graf et al., 2018)

Location	Per cent of MCT	Most common tumors
Hindlimbs	32,14	Soft tissue sarcomas (14,67%) Lipomas (13,44%) Hair follicle tumors (10,19%)
Flank	22,16	Lipomas (25,15%) Hair follicle tumors (15,27%) Soft tissue sarcomas (10,48%)
Chest / Thorax	20,58	Lipomas (42,22%) Soft tissue sarcomas (9,82%) Histiocytomas (6,67%)
Ventral Abdomen	20,25	Lipomas (35,54%) Vascular tumors (7,85%) Lymphoid tumors (5,79%)
Trunk	18,92	Lipomas (27,44%) Hair follicle tumors (13,89%) Soft tissue sarcomas (10,53%)
Extremities	17,63	Soft tissue sarcomas (17,75%) Histiocytomas (12,61%) Lipomas (9,81%)
Back	14,05	Hair follicle tumors (31,96%) Lipomas (7,99%) Vascular tumors (7,99%)
Forelimbs	13,84	Soft tissue sarcomas (25,11%) Histiocytomas (14,41%) Lipomas (12,98%)
Neck	12,84	Hair follicle tumors (24,32%) Lipomas (12,84%) Histiocytomas / Vascular tumors (each 9,14%)
Tail	10,45	Hair follicle tumors (41,04%) Histiocytoma (8,21%) Lipomas / Vascular tumors (each 7,46%)
Eyelid	10,36	Sebaceous tumors (25,00%) Melanocytic tumors (13,76%) Histiocytomas (6,42%)
Ear	10,36	Histiocytomas (33,25%) Hair follicle tumors (14,22%) Sebaceous tumors (9,16%
Head	7,91	Histiocytoma (20%) Sebaceous tumors (13,47%) Melanocytic tumors (12,32%)
Paws / Toes / Claws	7,07	Epidermal tumors (18,85%) Histiocytomas (15,98%) Soft tissue sarcomas (15,27%)
Perioral skin	6,39	Melanocytic tumors (20,00%) Histiocytomas (19,44%) Epidermal tumors (13,89%)

7. Treatment

Different treatment options are available for MCTs. While for 90% of low-grade MCTs, surgery completely cures the disease, adjunctive therapy is always suggested for high grade MCTs (Kiupel, 2017). The most common adjunctive therapies are done with irradiation, chemotherapy and tyrosine-kinase inhibitors. Literature suggests that in some cases, especially when resection of the tumor is not possible, these therapies can be used as primary treatment options or as cytoreductive measures.

Miller et al. (1981) defined the measurable conditions to evaluate the success of treatment. It can be characterized into complete response, partial response, no change and progression. Complete response describes the disappearance of all known disease in minimum two observations not less than 4 weeks apart. Partial response is defined as the decrease of tumor by 50% or more in two observations in not less than 4 weeks apart. No change is defined as less than 50% decrease and less than 25% increase in tumor. Progression of the disease describes 25% or more increase in lesions or the appearance of new lesions.

7.1. SURGERY

In localized, non-metastatic canine cutaneous MCTs, excision is the treatment of choice (Blackwood et al., 2012).

7.1.1. SURGICAL MARGINS

Earlier, a lateral margin of 3 cm and at least one fascial plane deep was suggested (Govier et al., 2003; London et al., 2003). More recent studies suggest that for grade I and II (Patnaik) / low-grade (Kiupel) MCTs smaller surgical margins are required for complete excision. Fulcher et al. (2006) reported complete excision in 91% (21/23) of cutaneous MCTs of grade I and II using a lateral margin of 2 cm and a deep margin of one fascial plane. Simpson et al. (2004) found 100% (3/3) of grade I and 75% (15/20) of grade II MCTs completely removed with a lateral margin of 1 cm. A 2 cm lateral margin was enough for complete removal in all grade I and II MCTs (23/23).

Pratschke et al. (2013) used modified proportional margins, meaning the largest tumor diameter was used as a lateral margin. In cases of a tumor diameter of more than 4 cm, a 4 cm lateral margin was used. 85% (40/47) of MCTs were successfully excised with clear margins. Itoh et al. (2021) found proportional margins to be enough for clean margins in 100% (25/25) of cases with low-grade MCT (tumor diameter ranged from 0,3-2,6 cm). Interestingly, one fascial plane deep excision failed to provide clean margins in 2 cases and

had close margins in 3 cases. Chu et al. (2020) compared wide margins (3 cm lateral margins) with another system of proportional margins. They used a 2 cm lateral margin for tumors bigger than 2 cm in diameter, below 2 cm they used the equivalent of the tumor diameter as a lateral margin. One deep fascial margin was done. No difference between wide margins (34/37; 92%) and the proportional system (43/46; 93%) was found, suggesting that the use of the proportional margins is safe.

In 2020, Saunders et al. did a retrospective study on the applicability of modified proportional margins for the excision of MCTs. It was found that 95% (95/100) were excised with clean margins, using the tumor diameter as lateral margins, tumors with a bigger diameter than 2 cm were excised with a 2 cm lateral margin. Deep surgical margin was one fascial plane. There was no association between tumor size or grade and complete excision and it was concluded that the modified proportional margins are a suitable technique for the excision of MCTs.

Séguin et al. (2001) concluded that complete excised grade II MCTs do not require further treatment after finding 95% (57/60) to not recur, with a median follow-up time 504 days (range: 77 to 1804 days).

For subcutaneous MCTs, excision alone was curative in tumors with complete margins in 98% of dogs (132/135) (Thompsen et al., 2011).

7.1.2. LYMPH NODES

In cases with confirmed or suspected lymph node metastasis, excision of the regional lymph node should be undertaken and follow-up irradiation should be considered (Blackwood et al., 2012). High grade MCT are more likely to have lymph node metastasis, so if cytology indicates the presence of a high grade MCT, the excision of the regional lymph node is highly suggested (Krick et al., 2009).

Intraoperative lymphoscintigraphy can be considered to find the sentinel lymph node. Worley et al. (2014) found 42% (8/19) of sentinel lymph nodes to not be the anatomically closest one by using intraoperative lymphoscintigraphy with blue dye. This suggests that regional metastasis could be missed in some cases with FNA alone. Ferrari et al. (2020) found sentinel lymph node mapping to be only working at first presentation as scar tissue altered the distribution of the radionuclide. In 63% (19/30) of dogs, sentinel lymph node was not the expected regional lymph node.

Recently, Fournier et al. (2020) suggested a new method for identification of sentinel lymph nodes. A list of possible sentinel lymph nodes is created and a contrast agent is

administered peritumoral. The possible lymph nodes are examined for the presence or absence of the contrast agent and thus, the sentinel lymph node can be identified and examined for metastasis.

7.1.3. MARGIN EVALUATION

Following excision, histological margin evaluation is suggested (Kiupel, 2017). In case margins are not clean, options are between re-surgery, radiotherapy or chemotherapy (Blackwood, 2012). Margins can be described as clean, close or dirty. Hayes et al. (2007) regarded margins as clean if they were excised with more than 10 mm into healthy tissue. Acceptable margins were between 5 to 10 mm and narrow margins within 5 mm to the tumor. In cases, the tumor exceeded the margin, the margin was regarded dirty. Séguin et al. (2001) define close margins are completely resected, but within 1mm range to the tumor. A histologically tumor free margin to prevent local recurrence has not been defined for MCTs yet. It was found that margins of less than 3 mm are sufficient to prevent local recurrence in low-grade MCTs, while in high-grade MCTs recurrence is independent of margins (Donnelly et al., 2013). Dores et al. (2018) concluded that low-grade MCTs with a histologic tumor-free margin of less than 10,9 mm should not be considered completely excised.

Dogs with incomplete excised grade II MCTs had a higher recurrence and metastases rate than completely excised grade II MCTs (Ozaki et al., 2007). Séguin et al. (2006) suggest that additional treatment after incomplete excision is not always necessary. Due to the negative prognosis of recurrence on survival, the recommendation is to evaluate immunohistochemical parameters (Ki67, mitotic count) in case of incomplete excision.

7.1.4. DOWNSTAGING (NEOADJUVANT TREATMENT)

Downstaging is a procedure used to reduce the size of tumors before surgery by administering chemotherapy or irradiation therapy, to increase the chances of full excision and thus improve the prognosis. McCaw et al. (1994) could show the reduction in size of MCTs after oral treatment with prednisone for 28 days in 20% (5/25) of dogs. Stanclift and Gilson (2008) could show 70% response rate to prednisone.

Olsen et al. (2018) did a retrospective study on neoadjuvant treatment prior to surgery. They used a combination of Toceranib and Vinblastine and it was administered for 4-16 weeks (median 6 weeks). 88% (14/16) of the dogs had measurable response to the treatment. 38% (6/16) dogs had complete response and 4 dogs even had gross resolution of their primary lesions, thus surgery was not performed after. Surgery was performed on the

other two dogs, histology did not show any sign of neoplasia throughout the tissue. Of all dogs that underwent surgery, in 70% (7/10) complete margins were achieved.

7.2.RADIATION THERAPY

Radiation therapy is based on ionizing radiation damaging DNA and thus inhibiting the cells' ability to divide and proliferate (Baskar et al., 2012). For incompletely resected MCTs as well as metastasis to the tributary lymph nodes, irradiation is the treatment of choice. Furthermore, irradiation is possible in cases of inoperable MCTs and for cytoreduction prior to resection (Kessler, 2012).

It should be noted that irradiation of MCTs can lead to degranulation of the mast cells and thus, adjuvant therapy in form of glucocorticoids or antihistamines should be administered to prevent local and systemic side effects (Dobson et al., 2004).

Multiple different therapeutic schemes are described in literature, using different amounts of Gray (Gy) and different intervals between irradiations. Continuous (daily fractions; Monday to Friday) and interrupted (alternate day fractions; Monday, Wednesday, Friday) fractions, as well as a 7-day interval is described for MCTs in literature. LaDue et al. (1998) compared continuous and interrupted irradiation fractions and found a significant longer disease-free interval for continuous fractions, thus it is the preferred option.

If radiation therapy is considered for the treatment of MCTs, it should be taken into consideration that veterinary clinics, that offer radiation treatment are rare due. Further, the treatment is expensive and time consuming (Hosoya et al., 2009).

7.2.1. IRRADIATION IN MCT WITH INCOMPLETE MARGINS

Only very few studies are available on the outcome of irradiation in relation the grade and stage of the tumor. All to me available studies are summarized in **Table 6**. Even though the results of these studies seem promising, it should be mentioned that there was no control group involved, therefore the outcome of MCTs without irradiation can only be assumed.

Weisse et al. (2002) found no local recurrence in grade 2, Stage 1 (complete excision, lymph node positive and excised) MCTs in 89% of the cases (24/27) and therefore do not suggest prophylactic irradiation therapy in these cases.

Table 6: Studies on irradiation of incomplete resected MCTs

Author	Dogs	MCT	Dosage	Outcome
Al-Sarraf et al. (1996)	32 dogs	Grade 2, Stage 0 Incompletely resected	18 fractions of 3Gy (54Gy) Alternate day fractions	94% 1-year survival 86% 2 to 5-year survival 100% 1-year disease free 96% 2 to 5-year disease free
Poirier et al. (2006)	45 dogs	Grade 2, Stage 0 Incompletely resected	15 fractions of 3,2Gy (48Gy) Daily fractions	80,6% 1-year disease free survival 67,1% 2- to 3-year disease free survival 94% 1- to 3-year local recurrence-free survival rate
Hahn et al. (2004)	31 dogs	Grade 3 Incompletely resected	18 fractions of 2,9Gy (52,2Gy) Alternate day fractions	Remission: 27 months median, 17 months mean (Range 1-47 months) Survival: 28-month median, 20 months mean (Range 1 to 52 months)

7.2.2.LYMPH NODE METASTASIS

Thamm et al. (2006) found prophylactic irradiation of the tributary lymph node to have a positive effect on the prognosis. Poirier et al. (2006) found no significant improve of disease-free or overall survival rate with prophylactic lymph node irradiation in a retrospective study including 45 dogs with grade 2 MCT.

Both studies were of retrospective nature, to estimate the usefulness of prophylactic nodal irradiation, a randomized, placebo-controlled study with a bigger population would be required.

7.2.3. INOPERABLE MCTS

Bulky tumors can show the occurrence of areas of radio-resistant tissue due to microenvironmental factors, such as hypoxia, and the possibility of occurrence of radiation-resistant tumor cell clones is higher and thus, these tumors should undergo cytoreductive treatment before irradiation (Blackwood et al., 2012). Dobson et al. (2004) described the treatment of non-resectable MCTs with irradiation and prednisolone in 35 dogs with an initial response rate of 88,5%. 1- and 2-year progression free rates were 60% and 52%, respectively. Prednisolone was started 10 to 14 days prior to the first irradiation. Irradiation was done using a 7-day interval in 4 fractions (Day 0, 7, 14, 21) of each 8 Gy (total irradiation dose: 32 Gy).

7.2.4. SIDE EFFECTS OF RADIATION THERAPY

Toxicity of irradiation therapy was described as low, dogs developed mild erythema, moist desquamation, if oral mucous membranes were involved mucositis but signs spontaneously resolved within 2–3 weeks. Late effects were seen in form of alopecia, hyperpigmentation and slight thickening of the treated skin (Dobson et al., 2004; Al-Sarraf et al., 1996).

7.3. GLUCOCORTICOID THERAPY

Glucocorticoids provide several therapeutic options in MCTs. They can be used to achieve a low-cost reduction of tumor burden in cases, where other therapeutic measures are not possible or not wanted by the owners. Further, they are involved in many therapeutic protocols as combination therapies to improve the outcome and can be used initially before surgical intervention to reduce the tumor volume and increase the chances of excision with clean margins. Glucocorticoids can prevent the release of histamine from mast cells and thus can be used to reduce clinical signs associated with MCTs (Löscher & Richter, 2016).

In a large, retrospective study on the systemic use of glucocorticoids, Elkholly et al. (2020) found side effects to occur in 4,9% of the dogs within 31 days of treatment. In cases where side effects occured, polydipsia accounted for 39,2%, polyuria for 28,4%, vomiting for 16,2% and diarrhoea for 14,9%. As glucocorticoids can have a negative effect on wound healing (Anderson & Hamm, 2014), this should be considered before using glucocorticoids prior to surgery. It is believed that due to the decrease in production of stem cell factor mediated by glucocorticoids, the growth, differentiation and chemotaxis of mast cells is consequently decreased (Stanclift & Gilson, 2008).

Matsuda et al. (2011) found a relation between the expression of glucocorticoid receptors in MCTs and its responsiveness to glucocorticoid therapy, suggesting that the expression of glucocorticoid receptors contributes to glucocorticoid sensitivity in MCTs and further suggests a direct effect of glucocorticoids in MCTs. Teng et al. (2012) reported a decreased response rate of MCTs with cytoplasmic Kit stain to prednisolone.

Takahashi et al. (1997) examined the effect of glucocorticoids (dexamethasone and prednisolone) on canine cutaneous MCTs. A significant inhibition and apoptotic-like cell death was seen. They concluded that grade I and II MCTs are more amenable to treatment with glucocorticoids than grade III MCTs. A possible explanation could be the loss of glucocorticoid-receptors in poorly differentiated tumors or the formation of cells with fewer or ineffective glucocorticoid-receptors.

Interestingly, intestinal MCT cells did not respond to glucocorticoid therapy, suggesting a difference in receptor expression.

McCaw et al. (1994) examined the use of oral prednisone as a sole treatment of grade II and III MCTs in 25 dogs. 20% (5/25) of the dogs showed a reduction in tumor size (4 partial and 1 complete remission). Oral prednisone was found to have an effect on MCT and can be considered as a adjuvant therapy, further a better response in lower grade MCTs was suspected. Stanclift & Gilson (2008) examined the use of prednisone as a neoadjuvant treatment in combination with surgical excision. The median diameter reduction was 45,2% and the reduction in tumor volume was 80,6%. No significant difference was found between the low dose (1 mg/kg) and high dose (2,2 mg/kg). Dogs were treated for 10 days prior to surgery. No consequences of pre-operative prednisone administration were found in any dog.

7.4. CHEMOTHERAPY

Chemotherapy should always be considered in grade III / high grade MCTs. Further indications are MCT, that are incompletely resected or not resectable (for control or down-staging), disseminated and metastatic MCTs and in cases, in which irradiation would be desirable but is not available or possible (Kiupel, 2017; Garrett, 2014; Blackwood et al, 2012; London et al., 2003). Often, chemotherapy is combined with prednisolone. Glucocorticoids alone can lead to remission of the MCT in 20–70% of cases, but this is most likely only of short duration, thus a combination therapy with chemotherapeutics is desirable (Kessler, 2012).

The usefulness of chemotherapy should always be weighted up with possible side effects (Kiupel, 2017) and chemotherapy should be avoided if children or pregnant women are living in the household.

7.4.1. VINCA ALKALOIDS

Vinca alkaloids (vincristine and vinblastine) are mitotic inhibitors. They function by binding to tubulin and consequently stopping the mitosis in the metaphase (Löscher & Richter, 2016). Adverse effects of these drugs are myelosuppression (leukopenia, thrombocytopenia), neurotoxicity and mucositis (Löscher & Richter, 2016). Vinblastine has been found to be more effective in the treatment of MCTs and thus is preferred over vincristine (Dobson & Scase, 2007).

7.4.1.1. VINBLASTINE

Rassnick et al. (2008) examined the efficacy of vinblastine as a single treatment for non-resectable grade II and III cutaneous MCTs in two different dosages (2 mg/m^2 and 3,5 mg/m^2) in 51 dogs with MCTs. At the dose of 2 mg/m^2, treatment with vinblastine did not

show sufficient response rate (12%; 3/25). Increasing the dose to 3,5 mg/m^2, the response rate was 27% (7/26) and thus was deemed promising for further investigations. The most common side effect observed was neutropenia (84% of dogs at 3,5 mg/m^2 of which 46% were severe), followed by gastrointestinal signs (35% of dogs at 3,5 mg/m^2). Due to prophylactic treatment with antibiotics, hospitalisation was only required in 8% (2/26) of dogs.

Davies et al. (2004) examined the use of vinblastine in combination with prednisolone in 27 dogs with grade II or III (Stage 0), incompletely excised MCT that have not undergone further treatment yet. It was tolerated well and within the following 12 months only two dogs had local recurrence. From the 206 dogs, which received this combination, only 13% showed adverse effects. Neutropenia was the most common (11%), followed by vomiting (4%) and diarrhoea (1%). Side effects were usually mild, only one dog died due to neutropenia and sepsis.

Hayes et al. (2007) examined the combination therapy with vinblastine and prednisolone on surgically excised grade III MCTs in 14 dogs. Side effects were observed in 5,6% of vinblastine doses (in 26% of dogs). Compared to surgical treatment alone, a modest increase of survival time was found, but to confirm the finding, a larger scale study would be required. Further, it was proposed that the combination therapy of excision, chemotherapy and irradiation could have a positive outcome on grade III MCTs.

Rungsipipad et al. (2009) examined the differences of treatment with vinblastine in combination with prednisolone compared to treatment with prednisolone alone in grade II MCTs. Vinblastine in combination with prednisolone showed a partial response of 78,2% (18/23) and the remaining 21,8% (5/23) remained stable, prednisolone alone showed a partial response in 50% (5/10), 30% (3/10) were stable and 20% (2/10) showed disease progression. Both protocols were concluded to be effective.

Thamm et al. (1999) compared the treatment with vinblastine and prednisone as an adjuvant treatment after excision and as a treatment option for gross disease. 57% (13/23) of the dogs treated as adjuvant therapy had 1- and 2-year disease-free interval. Of the dogs treated for gross disease, 47% (7/15) responded, of which two had a partial and five had a complete response. The median response duration was 154 days. Side effects of vinblastine and prednisone treatment usually occured after the first dose, only 20% (8/41) showed side effects, of which six cases were mild and two severe. Further, it was shown that the in grade III MCTs, the survival time was increased compared to surgery alone, with a median survival time of 331 days and 45% of patients alive after 1- and 2-years. The use of

vinblastine and prednisone was suggested as an adjuvant therapy after surgery and not only in case of recurrence.

In dogs with a combined AgNOR x Ki67 score of 54 or more, treatment with vinblastine and prednisolone was found superior compared to treatment with TKIs, given the absence of an ITD in exon 11 (Kiupel, 2017).

7.4.1.2. VINCRISTINE

McCaw et al. (1997) examined the use of vincristine for the treatment of MCTs and found it to be ineffective. Only 7% (2/27) of the treated dogs had a partial response present 4 weeks after the start of the treatment. Further, high toxicity was documented and led to discontinuation of treatment in 32% (9/27) of dogs. Compared to the sole treatment with prednisone, McCaw et al. (1997) saw no advantage and thus, does not recommend vincristine as a sole therapy for MCTs.

7.4.2.LOMUSTINE

Lomustine, also known as CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea), is a chemotherapeutic agent. It alkylates cytosine- and guanin-bases of the DNA and also reacts with proteins to inhibit DNA-repair and RNA-synthesis. A big advantage of the treatment with lomustine is the possibility to administer it orally. Common side effects associated with the administration of lomustine are myelosuppression, nausea, vomiting and hepatotoxicity. Rarely apathy and ataxia are possible (Löscher & Richter, 2016).

The first to describe the use of lomustine in MCT were Rassnick et al. in 1999. They examined its effect as a sole treatment on macroscopic MCTs in 19 dogs and found measurable response in 42,1% (8/19). One dog with a grade II MCT had a complete response for 440 days. Seven other dogs had a partial response with a median and mean duration of 77 and 109 days, respectively (range 21 to 254 days). Of these seven MCTs, one was grade I, two were grade II and four were grade III). Lomustine was found to be a considerable option in case of high grade MCT, recurrence or metastasis, especially if other treatment options are not available.

Hosoya et al. (2009) examined the outcome of treatment with lomustine and prednisone in a retrospective study including 15 dogs with incompletely resected grade II MCTs. They found no local recurrence or metastatic disease in any of the dogs. Progression-free time was 100% and 77% at 1-year and 2-years, respectively. Two dogs included in this study died of liver failure, otherwise this treatment protocol was tolerated well. Lomustine was concluded to be a viable alternative to irradiation therapy, but a bigger study with a

uniform therapeutic protocol would be required to obtain a better idea about the usefulness of lomustine.

Hay and Larson (2019) found lomustine in combination with prednisone to be a cost-effective alternative to other adjuvant treatment options for high grade MCTs. In a retrospective study they examined 15 completely excised high-grade MCTs and found recurrence in 13% (2/15) and metastasis in 13 (2/15) dogs. Nine dogs were alive after 1-year and six dogs after 2-years. Out of the 15 dogs, 13 had some sort of side effect during the treatment with neutropenia (67%) and elevation of alanin-aminotransferase (ALT) (60%) being the most common ones.

In a study examining the side effects of lomustine in a population of 185 dogs, neutropenia (56,9%), anaemia (34,2%) and thrombocytopenia (14,2%) were found due to the myelosuppressive effect of lomustine. Gastrointestinal signs occurred in 37,8% of dogs, most commonly presenting as vomiting (24,3%). Renal toxicity was found in 12,2% and ALT elevation in 48,8% of dogs. The incidence of hepatic failure was 1,2% (Heading et al., 2011).

A retrospective study examining the hepatotoxic properties of lomustine including 179 dogs treated for various tumors showed 6,1% (11/179) of dogs developing hepatic toxicity. The incidence of hepatic toxicity was higher with a higher dosis of lomustine (Kristal et al., 2004)

7.4.3.CHLORAMBUCIL

Chlorambucil is a chemotherapeutic agent acting by inhibiting the replication of the DNA. It can be administered orally and is cost effective with low toxicity (Taylor et al., 2009). Its side effects have described as myelosuppression with leukopenia, neutropenia, thrombocytopenia and gastrointestinal signs including nausea, vomiting and diarrhoea (Löscher & Richter, 2016).

Taylor et al. (2009) used a combination of chlorambucil and prednisolone to treat 21 dogs with MCTs, which were unsuitable for surgery or irradiation. 13 grade II, 6 grade III and 2 MCTs of unknown grade were included into this study. The response rate was 38% (8/21), of which complete response was achieved in three and partial response in five dogs. Nine dogs had static disease and in the remaining four, the MCT was progressing. The median progression free interval for the eight dogs with a response was 533 days. The outcome of the treatment was not related to the tumor grade or stage, but this could be an accidental

finding due to low numbers of dogs included into the study. No toxicity was found in any of the dogs.

7.5.CHEMOTHERAPEUTIC COMBINATIONS

Various combinations of chemotherapeutics and therapies have been suggested for the treatment of MCT and no "gold-standard" was established just yet. Depending on grade and proliferative factors, different treatment protocols can be used and are suggested (**Table 7**).

Table 7: Overview on combination therapies for macroscopic MCTs

Combination	**Source**	**Number of dogs in study**	**Response**
Toceranib + Vinblastin	Robat et al. (2012)	14	71% response rate - 14% total response - 57% partial response
Toceranib + Vinblastine + Prednisolone	Olsen et al. (2018)	29	90% response rate - 31% total response - 59% partial response
Toceranib + Lomustine	Burton et al. (2015)	41	46% response rate - 10% total response - 36% partial response
Toceranib + Prednisone + Irradiation	Carlsten et al. (2012)	17	76,4% response rate - 58,8% total response - 17,6% partial response
Vinblastine + Cyclophosphamide + Prednisone	Camps-Palau et al. (2007)	11	64% response rate - 46% total response - 18% partial response
Vinblastine + Lomustine	Cooper et al. (2009)	56	57% response rate - 24% total response - 32% partial response
Vinblastine + Lomustine + Prednisone	Rassnick et al. (2010)	17	65% response rate - 30% total response - 35% partial response
Vincristine + Cyclophosphamide + Hydroxyurea + Prednisolone	Gerritsen et al. (1998)	15	60% response rate - 27% complete response - 33% partial response

7.6. TYROSINE KINASE INHIBITORS

Tyrosine kinase inhibitors (TKIs) are functioning by inhibiting the intracellular signalling of the SCF-R (KIT). 15-40% of canine MCTs express a c-Kit mutation, which can be detected with PCR (periodic chain reaction) (Kessler, 2012; Welle et al., 2008). A present c-Kit mutation increases the chances of a TKI therapy to be effective against MCT, although the absence of c-Kit mutation does not rule out the therapeutic success with TKIs (Horta et al., 2017).

Resistance against TKIs was examined by Halsey et al. (2014). It was found out that resistance can occur due to secondary mutations of the target oncogene, consequently leading to reactivation or conformational changes. Further, the activation or overexpression of alternative signalling pathways and the upstream or downstream of the intended TKI target were named as possible causes.

7.6.1. IMATINIB

Isotani et al. (2008) found Imatinib mesylate effective on MCTs. In their study, 100% (5/5) of dogs with internal tandem duplication (ITD) positive c-Kit mutation on exon 11 responded to treatment and had beneficial response within 14 days (1 complete remission, 4 partial remission). Such ITD mutation occurs in approximately 20% of canine cutaneous MCTs (Kiupel & Camus, 2019). Furthermore, 31,25% (5/16) of dogs without ITD mutation were found to respond to treatment. Nakano et al. (2013) were able to show the effectivity of Imatinib mesylate in 2 dogs with other c-Kit mutations than ITD mutation.

Imatinib mesylate has been shown to be hepatotoxic (Isotani et al., 2008).

7.6.2. TOCERANIB

Toceranib is a tyrosine-kinase inhibitor, indicated in recurrent, non-resectable grade 2 and 3 MCTs (Halsey et al., 2014). Further, it was found out that Toceranib inhibits VEGFR2 (vascular endothelial growth factor receptor 2) and PDGFRβ (platelet-derived growth factor receptor beta). A measurable down-regulation of Kit-phosphorylation was found 8 hours after the first administration of toceranib (London et al., 2009).

In a double-blinded, placebo-controlled study, London et al. (2009) examined the efficacy of toceranib and found 37,2% (32/86) of the population to have a response to toceranib treatment (8,1% total response, 29,1% partial response). A study examining the response of toceranib in MCTs with and without c-Kit mutations, showed a greater response in MCTs with c-Kit mutation (69%), compared to MCTs with wild-type c-Kit mutations (37%). Overall response of toceranib was 42,8% (62/145) (Gil da Costa, 2015). Contrary to this,

Weishaar et al. (2017) found c-Kit mutation status to not predict treatment response to toceranib. No significant association between c-Kit mutation status and time to tumor progression or duration of response was found in a study of London et al. (2009).

The response rate of toceranib greatly varied between MCTs with and without ITD (Internal tandem duplication) mutation. MCTs with ITD mutation showed response in 69% (20/29) of cases, while MCTs without only responded in 36,8% (42/114). MCTs expression an ITD in c-Kit responded to toceranib in 90% of cases, while in dogs without c-Kit mutation the response rate was only 25% (London et al., 2009).

Side effects of toceranib were described as anorexia, diarrhoea and lethargy (London et al., 2009).

7.6.3. MASITINIB

Masitinib primarily is indicated as adjuvant therapy in cases of incomplete resected, not resectable, metastatic or primary multiple MCTs (Kessler, 2012; Gil da Costa, 2015). As mentioned above, the main mechanism of action is the inhibition of the intracellular signaling of the SCFR (KIT). Hahn et al. (2008) suspected a secondary mechanism of action, such as the inhibition of other protein kinases, as masitinib was not only effective in KIT-mutations, but also in wild-type forms of KIT. Masitinib was found to also target c-Kit, PDGFR (Platelet-derived growth factor receptor), lymphocyte specific kinase, fibroblast growth factor receptor 3, focal adhesion kinase (Gil da Costa, 2015).

Masitinib is administered per os and should be taken daily for minimum 6 months (Hahn et al., 2008).

Side effects of masitinib are common and are described to occur in 61,5% to 64,1% of dogs (Smrkovski et al., 2013; Grant et al., 2016). Most commonly, vomiting, diarrhoea and edema are described. Less commonly, protein losing nephropathy, haemolytic anaemia and neutropenia can occur (Hahn et al., 2008). Grant et al. (2016) found vomiting to be the most common side effect, it was observed in 15,4% (6/39) of dogs. Further, Grant et al. (2016) found serum alanine-transferase to increase in 23,1% of dogs (9/39) treated with masitinib.

Kuijlaars et al. (2021) examined the occurrence of proteinuria in dogs treated with masitinib and proposed to monitor the urine protein: creatinine ratio weekly for the first month of treatment, as usually this is where patients develop proteinuria. Ratios greater than 0,5 should be reassessed within a week, in patients with ratios greater than 2,

masitinib was discontinued. Proteinuria present before the treatment with masitinib was not shown to worsen with the administration of masitinib.

Smrkovski et al. (2013) found masitinib to have an overall response rate of 50% (13/26). Median survival time of dogs responding to masitinib was 630 days, compared to 137 days for dogs that did not respond to masitinib. The survival time for dogs treated with masitinib was not found to be in correlation with tumor grade or presence of metastasis. Grant et al. (2016) found the median survival time to be 159 days (range: 14 to 1339 days), this comparably low value can be explained due to the high rate of metastatic disease (54,3%) included in the study. 43,6% (17/39) of dogs had a complete response to masitinib, while 43,6% (17/39) had a partial response. Only in 5,1% (2/39) of dogs progression of the tumor could be shown. Hahn et al. (2010) could show a significantly increased survival rate compared to the placebo group in their follow-up study.

Gentilini et al. (2020) found the mutations p.Asn508Ile and p.Ala510Val in KIT exon 9 to be a cause of resistance to Masitinib and Vinblastine in one case of MCT. Further, it was suggested to examine cases of resistance to obtain a better understanding of resistance and effected genes and consequently improve therapeutic options and diagnostic tests.

7.7. OTHER THERAPEUTIC OPTIONS

Literature suggested various treatment options which were showing promising results in the treatment of MCTs and deserve further research and trials.

7.7.1. TIGILANOL TIGLATE

Tigilanol tiglate (TT) is a cellular signaling molecular, acting by activating protein kinase C. TT is used for the intratumoral treatment of non-metastatic MCTs. Intratumoral injection will result in localized inflammatory response and further disruption of tumor vasculature and and induction of tumor cell death by oncosis. Within 2 to 7 days, haemorrhagic necrosis and destruction of tumor mass occur, resolution of the resulting wound happens after 28 to 84 days after treatment (De Ridder et al., 2021). During the treatment, dogs must receive glucocorticoids (prednisone, prednisolone), H1-antagonists (diphenhydramine) and H2-antagonists (famotidine) to minimize the risk of degranulation reactions. TT is a treatment option for MCTs with a maximum size of $8cm^3$. It is usable for cutaneous and subcutaneous MCTs and should be considered in inoperable cases. It showed a clinical response of 75% within 24 days and no recurrence in 93% of dogs within 84 days (De Ridder et al., 2021).

Brown et al. (2021) treated 18 dogs with cytologically detected high-grade MCTs with TT. After the first injection, they reevaluated the dogs after 28 days and in case of recurrence, they did a second cycle of injection. Using this scheme, they achieved complete remission in 56% (10/18) of dogs, with 6 of them still being alive and recurrence free after two years.

7.7.2. INTRALESIONAL CORTICOSTEROIDS

Triamcinolone is a long-acting corticosteroid. When injected into MCTs, it reduces peritumoral inflammation and swelling as well as having a direct cytolytic effect on neoplastic mast cells. Intertumoral corticosteroids should be taken into consideration to reduce tumor size prior to surgery (Fan & Lorimier, 2005).

In a retrospective study on 5 dogs receiving intralesional triamcinolone without any adjuvant treatment or previous treatment, complete response was achieved in one and partial response in three dogs, the in the remaining dog the disease was stable. Thus, intralesional triamcinolone was suggested as a well-tolerated and effective treatment of not-resectable MCTs (Case & Burgess, 2018). It should be noted that no information on grade or stage on these MCTs was provided and a bigger study would be required to evaluate a more accurate efficacy rate.

7.7.3. IMMUNOTHERAPY

Immunotherapy has been identified helpful for several tumors in human and veterinary medicine. Bacillus Calmette-Guerin in connection with human chorionic gonadotropin was found useful by Henry et al. (2007) in the treatment of MCTs. The mechanism of action is believed to be by a bacterial cell wall derivative that stimulates antitumoral effect by increasing interleukin-18 (IL-18) and subsequently increases T-helper cell type-1 cytokines including interleukin-2 (IL-2) and interferon-γ.

In a study with 95 dogs, Henry et al. (2007) compared the effectivity of immunotherapy (Bacillus Calmette-Guerin) and chemotherapy (vinblastine) and concluded that tumor response rates were similar. It should be highlighted that in dogs receiving the immunotherapy, neutropenia occurred significantly less.

7.7.4. ONCOLYTIC VIROTHERAPY

Ilyinskaya et al. (2018) did a pilot study on the use of virus in the treatment of MCTs. Sendai virus was shown to have oncolytic characteristics. Attenuated strains are spreading in tumor tissues and kill malignant cells. In 2 from 6 cases in this study, the therapy led to

the clearing of the MCT without surgery. Further, larger scale studies are required to see the actual potential though.

In a study from 2013, Hwang et al. examined the usage of reovirus for the treatment of canine MCTs. It could be shown that canine MCT cell lines are highly susceptible to reovirus inducing apoptosis. Their study suggests further investigations into the effect of reoviruses with special care to the susceptibility of bone marrow-derived mast cell to reovirus.

7.7.5. INTRAREGIONAL DEIONISED WATER

In this method, the sensitivity of mast cells to changes in osmolarity is utilized, resulting in cellular swelling and membrane lysis (Fan & Lorimier, 2005). Based on a study including 99 dogs treated with excision and hypotonic solution, no recurrence was seen in 73,8% (73/99). Of these 99 dogs, 20 dogs had complete excision and only 5% had local recurrence (1/20), 79 dogs had incomplete excision with 31,6% (25/79) having local recurrence. 96% of recurrences (25/26) were attributed to grade II and III (Grier et al., 1995).

Contrary to this, Jaffe et al. (2000) were not able to show a benefit of adjunctive therapy with deionized water regarding survival time or recurrence compared to treatment by excision alone. Furthermore, Brocks et al. (2008) found no significant difference between treatment with hypotonic solution and placebo treatment.

7.7.6. PHOTODYNAMIC THERAPY

Photodynamic therapy is the local application of light of certain wavelengths to activate systemically delivered drugs. It is only suitable for small, superficial lesions and thus, surgery should if possible be preferred (Blackwood et al., 2012). According to Buchholz and Walt (2013), side effects are rarely observed for photodynamic therapy. Associated signs were hyperaemia, edema, cyanosis and pruritus. Further, alopecia and thinning of the skin of the treated area can occur. Depending on the size of the lesion and the associated inflammation, systemic anti-inflammatory and antibiotic treatment should be considered.

Frimberger et al. (1998) examined the effect of photodynamic therapy on various tumors, including a cutaneous and a periocular MCT. In the cutaneous MCT, complete response was achieved, but after four months, MCT recurrence at distant sides occurred and chemotherapy was started. The periocular MCT showed partial response and recurrence after 7 weeks. Generally, the local reaction was well tolerated in 87% (13/15) of all treated patients and normal exposure to sunlight was possible again after less than five days.

Increased side effects were seen in MCTs, possibly linked to the degranulation following photodynamic therapy.

7.7.7. ELECTROCHEMOTHERAPY

Electrochemotherapy is a combination of chemotherapeutic drugs (cisplatin or bleomycin) and the application of electric pulses to increase drug uptake of the tumor (Blackwood et al., 2012). Based on the ease of administration, low cost and lack of toxicities, Spugnini et al. (2006) identified it to be a good alternative in cases of incomplete margin resection.

Compared to surgical intervention, electrochemotherapy with cisplatin as a single therapy showed to be a promising alternative with comparable antitumoral effectiveness to surgery, especially in small MCTs (Kodre et al., 2009; Lowe et al., 2016).

7.7.8. BRACHYTHERAPY

Interstitial brachytherapy with Iridium-192 was examined by Northrup et al. (2004). Despite the small number of dogs (n=11) in the study, it was shown efficient in the treatment of MCT and it was suggested to make a larger scale study.

7.7.9. ELECTROGENE THERAPY

In a study with 8 dogs with 11 MCTs, Pavlin et al. (2011) examined the effects of intratumoral electrogene therapy. In this therapy, human interleukin-12 (IL-12) is injected into the MCT and after, electric pulses were delivered to the tumor using an electric pulse generator. IL-12 activates natural killer cells, induces IFN-y (Interferon gamma), inhibits angiogenesis and stimulates nitric oxide production. In 82% (9/11) of the MCTs, a reduction of tumor size was achieved. The reduced tumor volume ranged from 13% to 83% (median 50%).

7.8. ADDITIONAL THERAPY

Manipulation of MCTs can lead to the release of histamine due to degranulation of mast cells. A massive release of histamine can lead to severe systemic signs, such as hypotensive shock and death (London et al., 2003). The preoperative administration of diphenhydramine is suggested for bulky MCTs is suggested by Garrett (2014). Blackwood et al. (2012) mentioned the negative effect of histamine on wound healing and thus suggests the use of antihistamines for the excision of MCTs.

If systemic signs of MCTs are expected or suspected, the administration of H1-antihistamines can reduce the production of gastric acid and thus reduce the occurrence of gastric ulcers. Other possible treatment options are cimetidine, ranitidine and famotidine, as well as omeprazole (London & Seguin, 2003; Blackwood et al., 2012).

8. Prognostic factors

Prognostic factors can be useful in differentiating MCTs that require adjunctive treatment and MCTs in which sole surgical excision is enough. Current prognostic factors used include histologic grade, mitotic index (MI), KIT localization and c-Kit mutation status (Thamm et al., 2020). Further, Ki67 has been identified to be equally predictive for survival time as MI (Berlato et al., 2015).

8.1. TUMOR GRADE

Patnaik et al. (1984) found the 1.500 day survival time of MCTs to be 93% for grade I, but only 44% and 6% for grade II and III, respectively. Kiupel et al. (2011) found high-grade MCTs associated with a faster development of additional tumors or metastasis and a decreased survival time compared to low-grade MCTs.

Thamm et al. (2006) found grade II MCTs with nodal involvement or mucocutaneous location to have a better prognosis than grade III MCTs, thus suggesting the importance of histologic grade for the prognosis.

8.2. MITOTIC INDEX

The mitotic index is a parameter for cell proliferation and has been shown to be a strong predictor for the outcome of several neoplastic diseases. Van Lelyveld et al. (2015) found a high MI to be predictive for short survival times, but a low MI was not associated with a long survival. The use of a three-tier system for MI to predict survival was proposed (**Table 8**).

Table 8: The influence of MI on survival (van Lelyveld et al., 2015)

	1 year	**2 years**	**3 years**
MI <2	93,3 %	93,3 %	89,3 %
MI 2 to 7	68,5 %	61,9 %	47,1 %
MI >7	29,4 %	14,7 %	0 %

Romansik et al. (2007) found MI directly correlating with tumor grade in MCTs. Median survival time was significantly higher in MI ≤5 compared to MI >5 with 70 months and 2 months, respectively.

8.3. KI67

Ki67 is a cellular protein, found in proliferating cells and is directly associated with MCT grade and survival (Vascellari et al., 2012; Sakai et al., 2002), as well as tumor behaviour in all grades (Maglennon et al., 2008). It is detected using immunohistochemical stains and indicates all cycling cells (Kiupel, 2017). Ki67 can be useful for therapeutic guidance,

especially in grade II MCTs to separate cases that require adjuvant therapy from cases that do not (Maglennon et al., 2008; Scase et al., 2006). A Ki67 score below 1,8 was associated with a 3-year survival probability of 95%. Scores above 1,8 however had a 50%, 46% and 33% probability of survival for 1, 2 and 3-years, respectively without adjuvant treatment (Maglennon et al., 2008).

Smith et al. (2015) found incompletely excised MCTs with a low Ki67 and AgNOR score to not require additional treatment.

Berlato et al. (2018) found the combination of mitotic index, Ki67 and MCM7 (Minichromosome maintenance protein 7) to be more predictive than the parameter on their own. Further the use of Ki67 and MCM7 was suggested in grade II / low grade MCTs with low MI.

8.4. NOR (NUCLEOLAR ORGANISER REGION)

NOR are chromosomal segments that upon staining with silver (AgNOR) show small black dots in the nuclear area (Treré, 2000). AgNOR correlates with the speed of the cell cycle and is associated with the grade of MCTs, as well as survival time (Scase et al., 2006; Kiupel, 2017). In a study conducted by Bostock et al. (1989), 73% (11/15) of dogs died from tumor-related disease with AgNOR counts greater than 4,9, while only 33% (6/20) of dogs with counts between 1,7 and 4,8 died from tumor-related disease. Simoes et al. (1994) suggested a decreased survival time in AgNOR counts greater than 2,25.

Scase et al. (2006) associated low AgNOR scores with an increased survival time but concluded that the AgNOR score alone does not provide additional prognostic information compared to the histological grade.

A combined score from Ki67 and AgNOR was proposed. It can be calculated by multiplying AgNOR count per cell with the Ki67 index. A result ≥54 is associated with an increased incidence of MCT-related mortality (Smith et al., 2015). Further, results ≥54 are indicative of recurrence in incompletely excised MCTs and aggressive behaviour of completely excised MCTs, thus suggesting follow-up treatment (Kiupel, 2017).

8.5. PCNA (PROLIFERATING CELL NUCLEAR ANTIGEN)

PCNA is a co-factor of DNA-synthesis during the replication phase. It can be detected with immunohistochemistry (Scase et al., 2006; Kiupel, 2017). Simoes et al. (1994) were able to show a decreased survival time for MCTs with PCNA counts higher than 261 in 5 high power fields. Contrary to this, Scase et al. (2006) were not able to show a significant association between PCNA score and survival.

Webster et al. (2007) suggest the use of PCNA in combination with AgNOR and Ki67 to increase the usefulness of these parameters.

8.6. C-KIT MUTATION

Webster et al. (2006) found mutations of c-Kit to be a negative prognostic factor, associated with an increased incidence of recurrence and death. Further, the presence c-Kit mutation was linked to increased incidence of metastasis and higher tumor grade (Webster et al., 2006; Zemke et al., 2002).

Takeuchi et al. (2013) associated the presence of an internal tandem duplication (ITD) in exon 11 with a significant decreased progression-free survival and further concluded the demonstration of ITD in exon 11 to be a useful predictor of progression-free survival.

Brocks et al. (2021) concluded that a mutation on exon 8 is associated with good prognosis.

8.7.ABERRANT KIT EXPRESSION

In non-neoplastic mast cells, KIT is exclusively expressed in the cell membrane. Aberrant KIT expression describes the presence of KIT in the cytoplasm of a neoplastic mast cell (Kiupel et al., 2004). A significant correlation between the expression of cytoplasmic KIT and high histological grades was found by Gil da Costa et al. (2007).

Kiupel et al. (2004) identified three different KIT staining patterns:

Pattern 1: membrane associated staining

Pattern 2: focal to stippled cytoplasmic staining with decreased membrane-associated staining

Pattern 3: diffuse cytoplasmic staining.

KIT staining pattern 2 and 3 have been associated with decreased survival time and an increased incidence of local recurrence, as well as a more aggressive biological behaviour. In the presence of cytoplasmic staining, Kiupel et al. (2004) suggest the use of adjunctive treatment.

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