Histopathology and prognostic panels to aid in the diagnosis and management of canine mast cell tumors*

Mast cell tumors (MCTs) are the most commonly diagnosed malignant skin tumors in dogs.[1] The cutaneous form is the most common presentation, but MCTs can also arise in other locations such as the subcutis, mucocutaneous tissues, and viscera.[1] The clinical presentation, gross appearance, biologic behavior, and clinical course of canine cutaneous MCTs (CMCTs) vary widely. CMCTs may range from small, low-grade masses that are cured by surgical excision alone to locally aggressive, highly metastatic tumors that decrease quality of life and may result in euthanasia or death.[1–3]

While all canine MCTs are considered malignant, the clinical behavior can be viewed as a spectrum from less aggressive to more aggressive.

Histopathologic evaluation of a biopsy by a veterinary pathologist provides important diagnostic and prognostic information. This includes the microscopic appearance of the neoplastic cells and grade determination to help predict biologic behavior, as well as surgical margin assessment to determine completeness of excision. In addition to the routine microscopic evaluation, further insight into the biologic behavior of MCTs may be obtained by specialized testing, such as the mast cell tumor prognostic panels (MCT-PPs).

Prognostic panels

MCT-PPs are performed on the same tissue submitted for the initial diagnostic histopathology.

Components of the MCT-PPs:

- Mitotic count (MC): number of mitotic figures per 10 high-powered fields (previously known as mitotic index)
- AgNOR: Histochemical method that identifies the generation time (speed of cell cycle progression)
- Ki67: Immunohistochemical (IHC) method that identifies the growth fraction (actively dividing cells)
- Ag67: Combines measures of growth fraction and proliferation rate (AgNOR x Ki67)
- KIT: IHC method determines KIT protein localization within neoplastic cells
- *c-KIT*: Polymerase chain reaction (PCR) based method to assess the presence of *c-KIT* mutations in exon 8 and exon 11

Ordering information and components of each MCT-PP offered by IDEXX Reference Laboratories can be found at the end of this diagnostic update.

Cutaneous mast cell tumors (CMCTs)

CMCTs are defined by dermal involvement, with/without extension into the underlying subcutis and deeper structures.[1,2]

Grading information

The most widely used grading systems for CMCTs are the 3-tier Patnaik and the 2-tier Kiupel systems.[1,3] IDEXX pathologists apply both grading systems to all CMCTs. The Patnaik system designates CMCTs as grade I, II, or III based on depth of invasion, cellular atypia, granularity, nuclear features, mitotic count, and multinucleation.[2] The Kiupel system designates CMCTs as low or high-grade based on mitotic count, presence of multinucleation, bizarre nuclei, and karyomegaly.[4] The most relevant microscopic features and their prognostic implications are summarized in table 1.

Surgical margin evaluation

The prognosis of an MCT may be affected by whether an adequate tumor-free zone of tissue was obtained during surgical resection.[1] While the width of tumor-free zone that would consistently predict recurrence (or lack thereof) could not be determined, studies have provided general recommendations.[5–7] To improve the accuracy of margin measurement, biopsy specimens submitted to IDEXX marked as MCT on the accession form are stained prior to trimming. All IDEXX biopsy slides are scanned to create high-resolution digital images that enable our pathologists to review and interpret each case using accurate digital magnification and measurement tools. IDEXX histopathology reports for all neoplastic lesions (including MCTs) include surgical margin analysis and measurements. Table 1 summarizes the most relevant prognostic information regarding surgical margins.

When to consider an MCT-PP

Assessing the patient's needs and treatment options can be challenging when an MCT is diagnosed. The comprehensive histopathology report details the microscopic characteristics, grading, and surgical margins. Addition of an MCT-PP and other staging diagnostics may also be indicated on an individual basis. Recent studies show correlations between certain quantifiable attributes of CMCTs assessed by components of the MCT-PP and biologic behavior of the tumor.[1] Information obtained from an MCT-PP may help predict the clinical behavior of the CMCT in the patient and may influence decisions regarding the appropriate treatment plan for the patient.

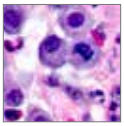
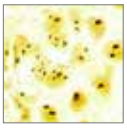
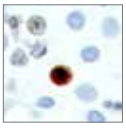
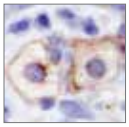
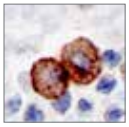
A complete MCT-PP (MCT-PP1) can be performed on almost any CMCT histopathology sample. There are some cases in which this would most impact treatment and management. Consider ordering this panel for a patient diagnosed with an MCT grade II (Patnaik system)/low-grade (Kiupel system) that presents with any of the following: rapid growth of the mass, a mass greater than 3 cm in diameter, anatomic location of concern. The complete panel (MCT-PP1) might also be helpful for MCTs that are intermediate in histologic appearance or have conflicting microscopic characteristics (e.g., low mitotic count, but significant nuclear atypia or multinucleated cells), making it harder to predict less aggressive versus more

aggressive behavior. Testing low-grade tumors (Kiupel system) may also be informative especially in cases of low-grade CMCTs that are marginally or incompletely excised, as Ki67 and AgNOR values help predict the likelihood of local recurrence, regardless of completeness of surgical excision. An unfavorable result on the complete MCT-PP1 for a low-grade tumor might encourage a more aggressive treatment approach, despite the histopathological assessment. Conversely, a favorable MCT-PP1 result might reassure the patient care team that a tumor with a less aggressive histologic appearance has been adequately treated with complete surgical excision alone.

For patients with grade III MCTs (Patnaik system) or high-grade MCTs (Kiupel system), cases of confirmed metastasis, or cases in which chemotherapy has been planned, adding the PCR component of the panel (MCT-PP3) may help to determine the likelihood that the patient will respond to a tyrosine kinase inhibitor (TKI).

The best means to identify low-grade CMCTs (Kiupel system) that may behave more aggressively are Ki67, Ag67, and KIT cellular localization pattern.[1]

Table 1. Overview of the most relevant prognostic information provided by routine diagnostic histopathology and MCT-PPs for CMCTs[1–16]

	Cutaneous mast cell tumors	
Mitotic count (previously referred to as "mitotic index")  Actively dividing neoplastic mast cell	• MC < 7: indicates a low-grade CMCT (Kiupel system): – MST: > 2 years – 5% of dogs died due to MCT-associated disease – 20% developed additional MCTs • MC ≥ 7: indicates a high-grade CMCT (Kiupel system): – MST: < 4 months – 90% of dogs died due to MCT-associated disease – 70% developed metastasis	• MC > 5: – MST of approximately 2 months (compared to 70 months in case of CMCTs with MC lower than 5) • 91% specificity of identifying aggressive CMCTs (with 79% diagnostic accuracy)
Surgical margins	• Grade I and II CMCTs (Patnaik system): – Tumors measuring < 3.2 cm excised with microscopic lateral SMs of at least 1 cm and a deep SM of at least 4 mm including a fascial layer did not recur • Low-grade CMCTs (Kiupel system): – In case of tumors measuring < 4 cm in diameter, 2 cm macroscopically determined lateral margins, and deep margins of one fascial plane resulted in complete excision – 96% of tumors did not recur, even though 29% were excised with microscopic margins of 3 mm or less	• High-grade CMCTs (Kiupel system): – General recommendation: macroscopically measured lateral margins of at least 3 cm and deep margins of one fascial plane – 36% recur locally despite histologically tumor-free margins
AgNOR  Cells with variable numbers of AgNORs (black dots)	• Average AgNORs per cell < 1.7: No dogs died due to MCT-associated disease • Average AgNORs per cell > 2.25: Significantly decreased survival • Average AgNORs per cell > 4: Significantly decreased survival: – 66.7% of dogs died from MCT-associated disease – MST 17 weeks	
Ki67  Ki67 positive cells (purple)	• Low-grade CMCTs (Kiupel system) with a low Ki-67 index and a low Ag67 are highly unlikely to recur, despite incomplete SMs: – Only 11% low-grade MCTs with a Ki67 ≤ 23 and Ag67 < 54 had local tumor recurrence • Average Ki-67-positive cells per grid area > 23: associated with increased disease progression and MCT-related mortality: – CMCTs with a low Ki-67 index rarely recur regardless of the cleanliness of the margins – CMCTs with a high Ki-67 index have a high risk to cause systemic disease	
Ag67 (AgNOR x Ki67)	• Best prognosticator for predicting decreased DFI • Associated with an increased risk of MCT-related mortality and metastasis • Ag67 > 54: significantly associated with an increased incidence and rate of MCT recurrence at the original surgical site: – 40% of dogs died due to MCT before 12 months postdiagnosis	• Ag67 is especially important for: – Incompletely excised low-grade CMCTs (Kiupel system): only 11% low-grade CMCTs with a Ki67 ≤ 23 and Ag67 < 54 had local tumor recurrence – Identification of low-grade CMCTs (Kiupel system) with more aggressive biological behavior than histologic grading alone would suggest
KIT protein localization		
 Pattern I: Perimembranous—associated with: – 2.4% rate of local recurrence – 14.3% rate of distant metastases – 2.4% mortality due to mast cell disease	 Pattern II: Focal/stippled—associated with: – 14% rate of local recurrence – 31% rate of distant metastases – 25.6% mortality due to mast cell disease	 Pattern III: Diffuse cytoplasmic—associated with: – 23.1% rate of local recurrence – 38.5% rate of distant metastases – 38.5% mortality due to mast cell disease – Significantly decreased DFIs and survival times compared to those with KIT pattern II
***c-KIT* mutations** • CMCTs with mutations in exon 11 tend to be more aggressive tumors • Therapy with TKIs has proven to be more effective against CMCTs with mutations in exon 11		

Abbreviation key: DFI: disease-free interval, **MC**: mitotic count, **MST**: median survival time, **SM**: surgical margin, **TKI**: tyrosine kinase inhibitors

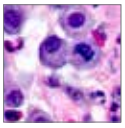
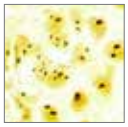
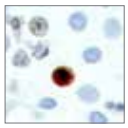
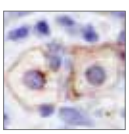
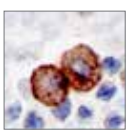
Subcutaneous mast cell tumors (SCMCTs)

SCMCTs are located within the subcutis, with no dermal involvement. It is important to distinguish these MCTs from CMCTs (located within the dermis with/without involvement of the underlying subcutis), as more than 90% of SCMCTs are controlled by surgical excision alone.[1] However, 10% of dogs with SCMCT die of MCT-related disease, 11% develop a second SCMCT distant from the primary site, 8% recur, and 5% metastasize.[1,9]

Grading information

While the Patnaik and Kiupel grading systems developed for CMCTs do not apply to SCMCTs, certain morphologic features (i.e., increased mitotic count, infiltrative growth pattern, and presence of multinucleated neoplastic cells) have been associated with a more aggressive biologic behavior.[8] The most relevant microscopic features and their prognostic implications are summarized in table 2.

Table 2. Overview of the most relevant prognostic information provided by routine diagnostic histopathology and MCT-PPs for SCMCTs[1,9,16]

	Subcutaneous mast cell tumors
Mitotic count (previously referred to as "mitotic index") 	• As compared to MC = 0, MC > 0 but ≤ 4 associated with: – 5.59 times higher risk of local recurrence – 3.72 times higher rate of MCT-related death • As compared to MC = 0, MC > 4 associated with: – 36.05 higher rate of MCT-related death – 130.21 times higher risk of local reoccurrence – Increased risk of and decreased time to metastasis
Presence of multinucleated cells	• Multinucleation was significantly associated with decreased survival times (hazard ratio = 3.40) • Associated with increased distant MCT occurrence rate by 2.24 times • Predicted MST: – Infiltrative tumor, MC > 4, and multinucleation: 140 days – Infiltrative tumor, MC > 4, but no multinucleation: 950 days
Growth pattern	• Infiltrative tumors: – 3.18 times higher rates of MCT mortality than well-circumscribed tumors – Significantly higher rates of MCT disease (local reoccurrence, distant MCT occurrence, and metastasis) than both circumscribed and combined patterns
Surgical margins	• In general, completely excised well-circumscribed SCMCTs are unlikely to recur • Recurrence rate: – With complete excision: 2% – With incomplete excision: 12% • Predicted time to local reoccurrence: – Incompletely excised SCMCTs with infiltrative pattern: 70 days – Completely excised SCMCTs with infiltrative pattern: 1,000 days – Incompletely excised well-circumscribed SCMCTs: 365 days
AgNOR 	• Average AgNORs per cell > 2.71: significantly more likely to locally recur than those with values lower than 2.71
Ki67 	• Average Ki67-positive cells per grid area > 21.8: predictive of a high risk of metastasis
Ag67 (AgNOR x Ki67)	• Ag67: used to identify the small subset of SCMCTs that exhibit a more aggressive biological behavior • Ag67 > 55: associated with an increased risk of MCT-related mortality and metastasis
KIT protein localization	
	Pattern I: Perimembranous—associated with: – Decreased odds of local recurrence and metastasis compared to pattern III – No significant difference compared to pattern II
	Pattern II: Focal/stippled—associated with: – Decreased odds of local recurrence compared to pattern III – No significant difference compared to pattern I
	Pattern III: Diffuse cytoplasmic—associated with: – Increased odds of local recurrence and developing metastasis compared to pattern I – Increased odds of local recurrence compared patterns I and II

Abbreviation key: MC: mitotic count, **MST**: median survival time

Surgical margin evaluation

As a general rule, if a SCMCT is well circumscribed and excision was complete, then the tumor is unlikely to recur.[1,9] A study showed 2% recurrence rate with complete excision and 12% recurrence rate even with incomplete excision.[9] Risk factors for local tumor recurrence of SCMCTs include mitotic count greater than 0, incomplete margins, and infiltrative growth pattern.[9] The predicted time to local reoccurrence for cases having incomplete margins and infiltrative pattern is 70 days, compared to 1,000 days for completely excised infiltrative tumors, and 365 days for incompletely excised well-circumscribed tumors.[1,9]

When to consider an MCT-PP

A complete MCT-PP (MCT-PP1) can be performed on almost any SCMCT histopathology specimen. Unlike CMCTs, location and size of SCMCTs have not yet been correlated with poor clinical outcome. Components of the MCT-PP have been shown to predict aggressive clinical behavior in SCMCTs, including likelihood of local recurrence of incompletely or narrowly excised SCMCTs as well as risk of metastasis. After assessing results of the histopathology, clinical behavior of the SCMCT, and client goals, the MCT-PP may provide valuable information to help guide treatment decisions including whether to consider re-excision of a SCMCT, radiation therapy, and/or chemotherapeutic intervention.

The best means to identify low-grade CMCTs (Kiupel system) that may behave more aggressively are Ki67, Ag67, and KIT cellular localization pattern.

Conclusions

Utilization of all aspects of the histopathology, including the microscopic description, grading, margin evaluation, and assessment of the MCT-PP, helps predict the clinical behavior of CMCTs and SCMCTs. Early identification and characterization of these tumors facilitate improved education of the pet owner, contribute to complete staging, and encourage development of an informed treatment plan for optimal patient care.

Utilization of all aspects of histopathology helps predict the clinical behavior of CMCTs and SCMCTs on the spectrum of low to high risk.

Low risk — High risk

Ordering information

Test code	Test name and contents
3375	**Mast Cell Tumor Prognostic Panel 1—Canine** Immunohistochemistry testing KIT and Ki67, histochemical staining for AgNOR, PCR analysis for mutation in exon 8 and exon 11 of the *c-KIT* gene and interpretation. **Note:** Must have ordered a prior or concurrent biopsy. **Specimen requirements:** Original tissue blocks **Turnaround time:** 7–15 working days
3373	**Mast Cell Tumor Prognostic Panel 2 (Stains)—Canine** Immunohistochemistry testing for KIT and Ki67, histochemical staining for AgNOR and interpretation. **Note:** Must have ordered a prior or concurrent biopsy. **Specimen requirements:** Original tissue blocks **Turnaround time:** 7–10 working days
3374	**Mast Cell Tumor Prognostic Panel 3 (PCR)—Canine** PCR analysis for mutations in exon 8 and exon 11 of the *c-KIT* gene and interpretation **Specimen requirements:** Original tissue blocks **Turnaround time:** 7–15 working days

Contacting IDEXX

In the United States: 1-888-433-9987

In Canada: 1-800-667-3411

For questions regarding specimen submission, please contact our Laboratory Customer Support Team.

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*This diagnostic update is intended to complement the information provided in the CMCT, SCMCT, and MCT-PP reports.
†Includes references cited in the CMCT, SCMCT, and MCT-PP reports.