FACT SHEET 1

Finn Pathologists *The* Veterinary Pathologists

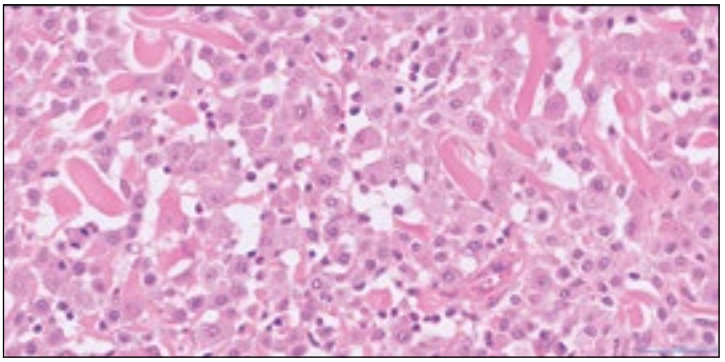
Melanie Dobromylskyj BSc Vet Path (Hons), BVSc, PhD, FRCPath, FRCVS

Canine cutaneous mast cell tumours – supplemental testing on histopathological samples

There are a plethora of additional tests available to aid further prognostication of canine cutaneous mast cell tumours (MCTs). These are offered either alone or in various combinations and all measure slightly different things, which can be a little bewildering.

This fact sheet aims to summarise the tests available, describing what they measure and how useful they are in terms of prognosis. There is currently no single individual marker which will reliably differentiate between the relatively benign majority of tumours from the more malignant and potentially fatal minority, therefore these prognostic markers must always be considered in conjunction with the histological grading, other markers, clinical staging and any additional clinical information such as tumour location. Please note this fact sheet only applies to canine cutaneous MCTs and is not for those arising in the subcutis, at other sites such as the oral cavity, or in other species.

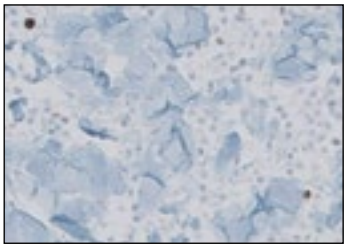
Consultation with a clinical oncologist is always advisable if considering further tests and/or treatment options for these tumours.



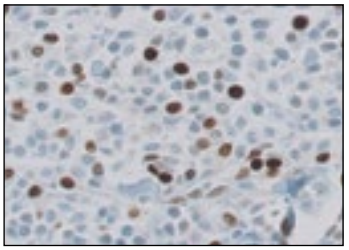
Mast cell tumour, from the skin of a dog, haematoxylin and eosin stain, high power (400x)

Ki67

Ki67 is a nuclear protein expressed in all active phases of the cell cycle but which is absent in non-cycling cells. It is detected by immunohistochemical (IHC) staining. The relative number of Ki67-positive cells indicates the growth fraction, or the relative number of cells actively involved in cell cycle growth at that particular point in time. It is possible to divide mast cell tumours into two groups based on the Ki67 score; those with higher Ki67 scores have a tendency for shorter survival times compared to those with lower scores. Ki67 has been shown to be a prognostic indicator independent of histological grade, meaning it provides further prognostic information. This test is available on its own, in conjunction with AgNOR or as part of the MCT Prognostic Profile.



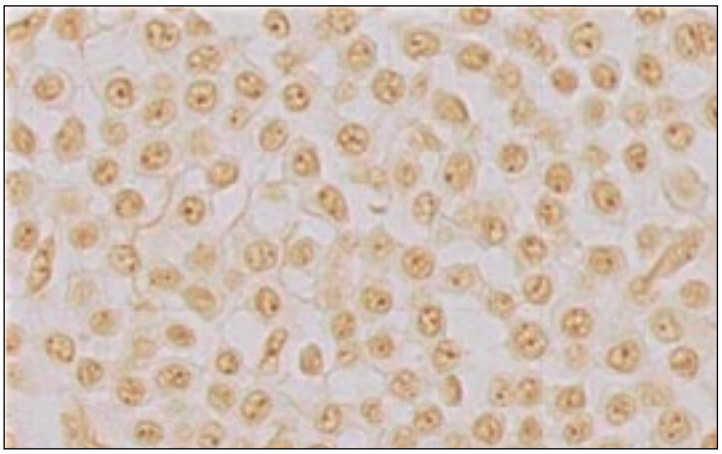
Mast cell tumour with a low Ki67 score; positive staining nuclei are coloured brown, against the blue counterstain (haematoxylin).



Mast cell tumour with a high Ki67 score; note the higher number of positive staining (brown) nuclei compared to the low scoring tumour.

AgNOR

AgNOR (argyrophilic nucleolar organiser regions) are areas within the nucleus associated with proteins involved in ribosomal RNA transcription. AgNORs are detected by a histochemical (silver) stain, and the number of AgNORs present within each nucleus is proportional to the rate of cell proliferation in vivo. Therefore the AgNOR count gives an indication of generation time, a component of the rate of cellular proliferation. Higher AgNOR counts in MCTs are associated with increased mortality, local recurrence and metastasis. Lower AgNOR counts correlate with longer survival times but are not predictive of clinical behaviour independent of histological grade; this reduces the usefulness of the test since it does not provide prognostic information additional to that already provided by the histological grade. Hence this test is only available in conjunction with Ki67 or as part of the MCT Prognostic Profile.



A mast cell tumour with an AgNOR stain. The AgNORs are small dark brown dots within the yellow nuclei.

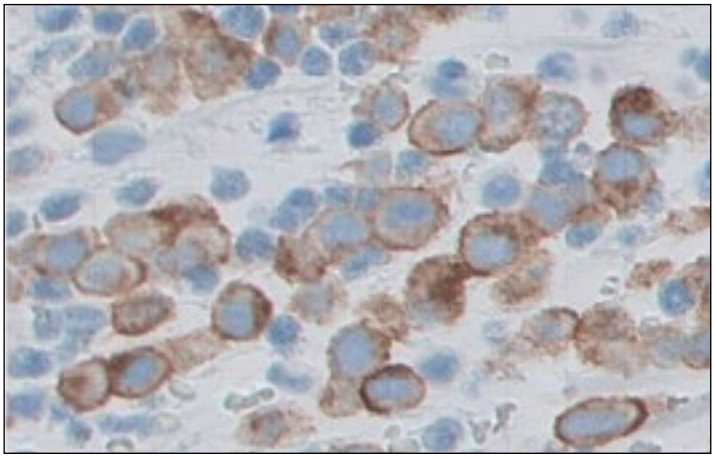
Combining Ki67 and AgNOR

Cellular proliferation is a product of both growth fraction and generation time, and so Ki67 and AgNOR scores are sometimes combined (also referred to as the Ag67 index) to give an indication of overall cellular proliferation within a tumour. The growth fraction and generation times are independent of one another, and thus are providing complementary biological information about the tumour cells, which may be more useful when the two indices are used in concert rather than individually.

c-KIT mutation

c-KIT is a proto-oncogene; these are normal genes involved in regulating cell growth and differentiation. *c-KIT* encodes a tyrosine kinase receptor (KIT), which is normally expressed on the cell surface and acts as a receptor for a growth factor. Mutations in the *c-KIT* gene convert it to an oncogene; these are genes that are abnormally activated and promote autonomous cell growth in cancer cells. Such mutations in the *c-KIT* gene can result in a KIT receptor which is permanently activated even in the absence of its ligand. *c-KIT* mutations can be an important contributory factor in MCT development and growth, for example by increasing cellular proliferation. *c-KIT* gene mutations are detectable by PCR, and some are associated with a worse prognosis. Knowledge of their presence may also influence the choice of chemotherapeutic agents, particularly the tyrosine kinase inhibitors. This test is available on its own as well as part of the MCT prognostic profile.

KIT staining patterns



A mast cell tumour stained for KIT using immunohistochemistry; these mast cells demonstrate positive (brown) staining consistent with a pattern I (membrane-associated).

KIT is the tyrosine kinase receptor encoded by the *c-KIT* gene, as discussed above. Normally, the KIT receptor is present on the cell surface (i.e. membrane-associated), and several studies have looked for changes in KIT staining patterns in tumour cells using IHC, and whether these are associated with outcome. Staining pattern I is membrane-associated staining with little or no cytoplasmic staining (i.e. normal), while staining pattern II is intense focal or stippled cytoplasmic staining, and pattern III is diffuse cytoplasmic staining of neoplastic mast cells. Staining patterns II and III, i.e. increased cytoplasmic staining of KIT, are thought to be associated with shorter overall survival times and increased risk of local recurrence. The histological grade, *c-KIT* mutations and KIT staining patterns are all independent factors, thus all can theoretically provide useful prognostic information in their own right. This test is available as part of the MCT prognostic profile.

Please note that these additional tests for mast cell tumours rely on there being an adequate number of identifiable mast cells present in the sample for accurate assessment. Also for incisional biopsies, it is assuming that the sample submitted is fully representative of the mass as a whole.

Hence, these tests become less accurate in the following circumstances:

- Small samples, with low numbers of mast cells (small incisional biopsies)
- Samples with artefact, such as crush or cauterization, where the mast cells cannot be accurately identified
- Mast cell tumours which are overrun by eosinophils
- Mast cell tumours with necrosis and/or significant inflammation

If you require any further assistance in understanding the further prognostic tests available for mast cell tumours please do not hesitate to contact us.

Recommended reading:

Bellamy E., Berlato D.J. (2022) “Canine cutaneous and subcutaneous mast cell tumours: a narrative review.” Journal of Small Animal Practice. 63(7): 497-511.

Blackwood, Murphy et al. (2012) “European consensus document on mast cell tumours in dogs and cats.” Veterinary and Comparative Oncology issue 10, e1 - e29.

Finn Pathologists
Unit 3C & 3D Mayflower Way • Harleston • Norfolk • IP20 9EB
Tel: 01379 854180 • email@finnpathologists.com • www.finnpathologists.com