

## BRAF mutation in canine transitional cell and prostate carcinoma

### Transitional cell and prostate carcinoma

In dogs, both transitional cell carcinoma (TCC, Fig. 1) and prostate carcinoma (PCa) are highly malignant neoplasms (GRIFFIN et al. 2018). Both types of tumour are often **diagnosed** rather **late** (BRYAN et al. 2007; PANTKE 2018). Consequently, prognosis for affected dogs is bad (CORNELL et al. 2000; HENRY 2003). The **median survival time** is one year for dogs with TCC and 30 days for dogs with PCa (HENRY 2003; CORNELL et al. 2000). The median age of affected dogs is 11 (TCC) and 10 years (PCa) (CORNELL et al. 2000; KNAPP et al. 2000). For a long time, there was no reliable screening test for the presence of TCC, particularly for breeds with increased genetic risk (certain terrier breeds).

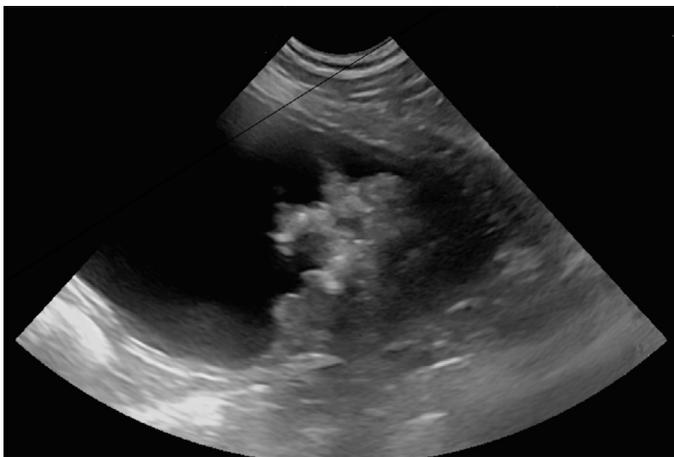


Fig. 1: Sonographic image of the urinary bladder. A papillary mass clearly protrudes into the lumen of the bladder.

©Tierklinik Rupphübel

### BRAF mutation

The presence of BRAF variant V595E, known from human medicine, was studied for the first time on a large number of tumours in dogs by MOCHIZUKI et al. in 2015. In contrast to people, where the mutation occurs mainly in malignant melanoma, ovarian tumours and colorectal carcinoma, in dogs, MOCHIZUKI et al. (2015) most often found the mutation in **transitional**

**cell and prostate carcinomas**. It is a somatic **mutation** in the **BRAF gene** which only occurs in the **tumour cells**. It is assumed that this mutation leads to **tumour development** through permanent activation of the MAP kinase pathway.

### BRAF mutation in canine transitional cell carcinoma

In 2018, we succeeded in establishing the method for detecting BRAF mutation in TCC in our laboratory (AUPPERLE-LELLBACH et al. 2018). Specificity was 100% as the BRAF mutation could not be detected in any of the dogs with cystitis, urinary bladder polyp or in those without any clinical and pathological signs. Sensitivity of BRAF mutation detection in TCC was 71%.

Possible sample materials:

- tissue (e.g. biopsies)
- cytological smears (e.g. FNA)
- urine rich in cells (early morning urine)

In the literature, the test has so far only been described for tissue samples and urine. In our studies, we have also succeeded in **establishing** it for **cytological smears** of fine needle aspirates and urinary sediments.

Thus, **invasive sampling** can be **avoided** by testing spontaneous urine, or there is **no repeated invasive sampling necessary** in cases of **questionable histopathological and cytological diagnoses** (poor sample quality, overlapping images of inflammation and neoplasia).

A **breed predisposition** has been described for canine **TCC** in Scottish, Fox and West Highland White **Terrier** (FULKERSON und KNAPP 2015). Another study also showed a predisposition

for BRAF mutation in different long- and short-legged terriers (AUPPERLE-LELLBACH et al. 2019). It can thus be concluded that detection of **BRAF mutation** in these terrier breeds is not only **highly specific** for the presence of TCC, but also **very sensitive** (Fig. 2) and can be used as a **screening test** for the presence of TCC.

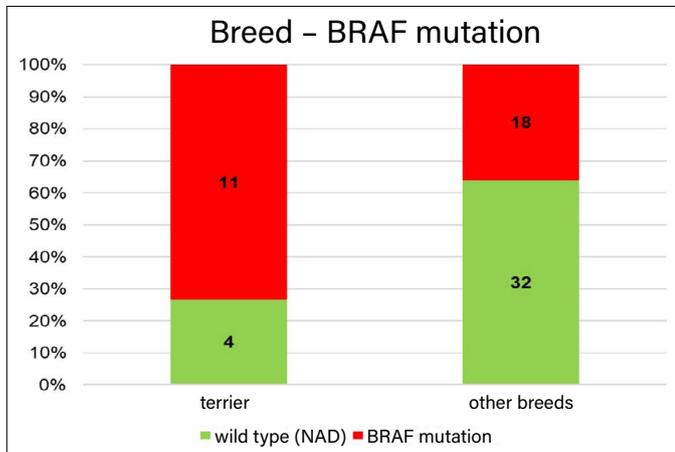


Fig. 2: Correlation between BRAF mutation and dog breed. Terriers were significantly more likely to show BRAF mutation in transitional cell carcinoma than other breeds.

No gender or age predisposition for BRAF mutation could be shown in this study. There was also **no correlation** between the detection of **BRAF mutation** and the **invasivity** or **aggressiveness** of TCC in dogs (GRASSINGER et al. 2019).

In a small study (n=10), **carcinomas of the kidney** tubules did not show BRAF mutation. However, two of six carcinomas of the transitional epithelium of the renal pelvis were positive (AUPPERLE-LELLBACH et al. 2019).

### BRAF mutation in canine prostate carcinoma (PCa)

For canine PCa (Fig. 3) as well, detection of BRAF mutation from different materials could be established (GRASSINGER et al. 2019).

Possible sample materials:

- *tissue (e.g. biopsies)*
- *cytological smears (e.g. FNA)*

Again, **specificity** was **100%** as the mutation could neither be detected in samples with benign prostatic hyperplasia, squamous epithelial metaplasia, atrophy of the prostate nor in normal prostate tissue.

**Sensitivity of BRAF mutation detection** in canine PCa was **61%**.

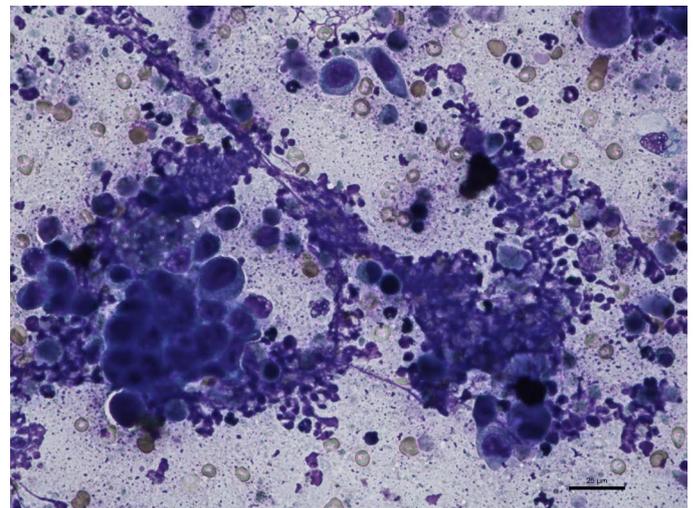


Fig. 3: Cytological picture of a prostate carcinoma with prostatitis: numerous partly degenerated neutrophil granulocytes and clusters of pleomorphic prostate epithelial cells with large, differently shaped cell nuclei (Haema Quick Stain, bar = 25 µm)

Based on a histological score, **prostate carcinomas caused by BRAF mutation** (Fig. 4) were much more **aggressive** than those not caused by this mutation (GRASSINGER et al. 2019).

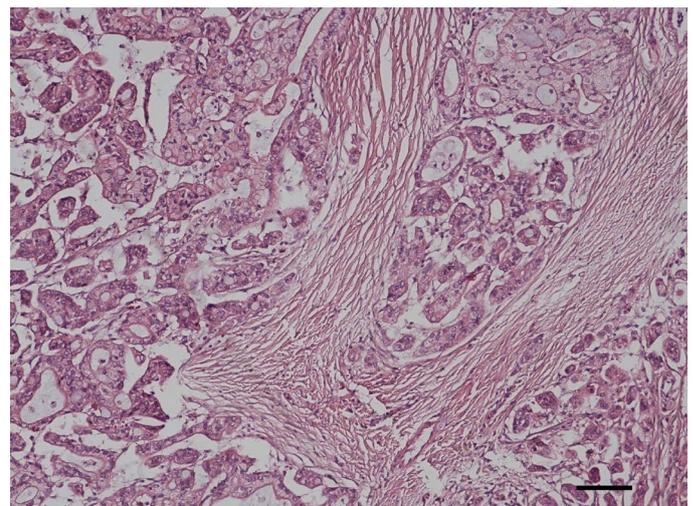


Fig. 4: Histological picture of a highly aggressive, tubulopapillary prostate carcinoma with infiltrative growth (Gleason Pattern 3, score sum 8, bar = 100 µm)

### Conclusion

BRAF mutation testing is a **highly specific** method (**100%**) for the detection of canine transitional cell and prostate carcinoma.

**Sensitivity**, however, varies depending on the site (kidney, urinary bladder, urethra, prostate gland) and possibly the breed:

*Transitional cell carcinoma:*  
86% long- and short-legged terrier  
44% other breeds

*Prostate carcinoma:* 60%

Testing for BRAF is **indicated** in cases in which

- invasive sampling should be avoided,
- the cytological/histological result is not conclusive.

•  
Only a **positive result** confirms a carcinoma.

If no **BRAF mutation** is detected in the sample, it may be because of the following reasons:

- There is no TTC/PCa (e.g. polyp, benign hyperplasia).
- The carcinoma is not caused by the BRAF gene mutation.
- There are no mutated cells in the sample, even though a carcinoma is present (representativeness of the sample, cytology/urine low in cells).

### **BRAF mutations in feline urinary bladder carcinoma**

We examined biopsies of 25 cats with TCC (n=19), urinary bladder polyp (n=2) or cystitis (n=4) for the presence of BRAF mutation (HOHLOCH et al. 2019). There was no mutation detected in any case!

Thus, BRAF mutation analysis is **not suitable for** the diagnosis of transitional cell carcinoma in **cats**.

### **Literature**

- AUPPERLE-LELLBACH H, GRASSINGER J, HOHLOCH C, KEHL A, PANTKE P (2018): Diagnostische Aussagekraft der BRAF- Mutation V595E in Urinproben, Ausstrichen und Biopaten beim kaninen Harnblasen- karzinom. *Tieraerztliche Praxis Kleintier*. 46: 289–295.
- AUPPERLE-LELLBACH H, KEHL A, MERZ S, GRASSINGER J, HOHLOCH C, PANTKE P (2019): Die BRAF-Mutation V595E im Übergangszellkarzinom – Untersuchungen zur Rassedisposition bei Terriern. *Kleintier- medizin*. 1: 30–33.
- AUPPERLE-LELLBACH H, GRASSINGER J, ERHARD H, KEMPKER L, MERZ S, PANTKE P (2019): BRAF-mutation in carcinomas of various sites in the canine urinary tract. Abstract ECVIM 2019 in Milan, Italy
- BRYAN JN, KEELER MR, HENRY CJ, BRYAN ME, HAHN AW, CALDWELL CW (2007): A population study of neutering status as a risk factor for canine prostate cancer. *Prostate*. 67: 1174–1181.
- CORNELL KK, BOSTWICK DG, COOLEY DM, HALL G, HARVEY HJ, HENDRICK MJ, PAULI BU, RENDER JA, STOICA G, SWEET DC, WATERS DJ (2000): Clinical and pathologic aspects of spontaneous canine prostate carcinoma: A retrospective analysis of 76 cases. *Prostate*. 45: 173–183.
- FULKERSON CM, KNAPP DW (2015): Management of transitional cell carcinoma of the urinary bladder in dogs: a review. *Veterinary Journal*. 205: 217–225.
- GRASSINGER JM, MERZ S, AUPPERLE-LELLBACH H, ERHARD H, KLOPFLEISCH R (2019): Correlation of BRAF Variant V595E, Breed, Histological Grade and Cyclooxygenase-2 Expression in Canine Transitional Cell Carcinomas. *Veterinary sciences*. 6: doi: 10.3390/vetsci6010031.
- GRASSINGER JM, AUPPERLE-LELLBACH H, ERHARD H, MERZ S, KLOPFLEISCH R (2019): Nachweis der BRAF-Mutation bei kaninen Prostataerkrankungen. *Tieraerztliche Praxis Kleintier*. 2019; 47: 1–8
- GRIFFIN MA, CULP WTN, REBHUN RB (2018): Lower Urinary Tract Neoplasia. *Veterinary sciences*. 5: doi:10.3390/vetsci5040096
- HENRY CJ (2003): Management of transitional cell carcinoma. *The Veterinary clinics of North America. Small animal practice*. 33: 597–613.
- HOHLOCH C, KEHL A, AUPPERLE-LELLBACH H (2019): BRAF Variant V637E is not detectable in biopsies of feline transitional cell carcinoma. Abstract ESVCP-ECVP Congress, Arnhem, the Netherlands
- KNAPP DW, GLICKMAN NW, DENICOLA DB, BONNEY PL, LIN TL, GLICKMAN LT (2000): Naturally-occurring canine transitional cell carcinoma of the urinary bladder, a relevant model of human invasive bladder cancer. *Urologic Oncology: Seminars and Original Investigations*. 5: 47–59.
- MOCHIZUKI H, KENNEDY K, SHAPIRO SG, BREEN M (2015): BRAF Mutations in Canine Cancers. *PLoS one*. 10: e0129534.
- PANTKE P (2018): Diagnostik und Therapie des Übergangszellkarzinoms des unteren Harntraktes beim Hund. *Kleintierpraxis*. 63: 76–92.