**Supplemental Data**

**Supplemental Table 1.**

Excel spreadsheet containing all pass-filter somatic mutations from the four InvTCC tumors.

**Supplemental Table 2.** Genotyping results for all samples referenced in the text.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Tumor | Germline | Urine | Cell line |
| AUSS\_220 | AA | TT |  |  |
| AUSS\_877 | TTa |  | TTa |  |
| BEAG\_989 | ATc |  |  |  |
| BEAG\_463 | AT | TT |  |  |
| BEAG\_763 | AT |  |  |  |
| BEAG\_927 | ATb | TT |  |  |
| BEAG\_391 | AT | TT |  |  |
| BEAG\_214 | AT | TT |  |  |
| BEAG\_887 | AT | TT |  |  |
| BEAG\_512 | AT | TT |  |  |
| BEAG\_519 | AT |  | AT |  |
| BEAG\_314 | AT |  | ATb |  |
| BICH\_489 |  |  | TT |  |
| COLL\_730 |  | TT |  | TT=K9TCC\_Sh |
| DACH\_231 | AT | TT |  |  |
| DACH\_797 | AT |  | AT |  |
| DACH\_301 |  |  | AT |  |
| FOXT\_608 | AT |  |  |  |
| FOXT\_713 | AT | TT |  |  |
| GSD\_765 |  | TT |  | AT=K9TCC\_Mx |
| GSD\_721 | AT | TT |  |  |
| GSD\_701 | AT | TT | AT |  |
| GSHP\_901 | TTa |  |  |  |
| GSHP\_317 | ATc |  |  |  |
| JACK\_714 | AT | TT |  |  |
| JACK\_795 | AT |  |  |  |
| JACK\_456 | AT |  |  |  |
| MINP\_540 | ATc | TT |  |  |
| MINP\_700 | ATb | TT |  |  |
| MSNZ\_415 | ATb | TT |  |  |
| MIX\_501 |  | TT |  | ATa =K9TCC\_Pu |
| MIX\_493 |  |  |  | AT=K9TCC\_AxA |
| MIX\_904 | AT | TT |  |  |
| MIX\_695 | AT | TT |  |  |
| MIX\_223 | TTc | TT |  |  |
| MIX\_038 | TTa | TT |  |  |
| MIX\_652 | AT | TT |  |  |
| MIX\_719 | AT | TT |  |  |
| MIX\_878 | AT | TT |  |  |
| MIX\_879 | AT | TT |  |  |
| MIX\_568 | AT | TT |  |  |
| MIX\_854 | AT | TT |  |  |
| MIX\_082 | AT | TT |  |  |
| MIX\_174 | AT | TT |  |  |
| MIX\_707d | AT |  | ATb |  |
| MIX\_366 | TTa |  | TTa |  |
| MIX\_179 | AT | TT |  |  |
| MIX\_276 | AT |  | AT |  |
| MIX\_552 |  |  | TT |  |
| MIX\_596 |  |  | AT |  |
| MIX\_022 | TTa |  | TTa |  |
| SAMO\_502 |  |  |  | AT=K9TCC\_Nk |
| SCOT\_140 | AT | TT |  |  |
| SCOT\_353 | ATb | TT |  |  |
| SCOT\_731 | AT | TT |  |  |
| SCOT\_843 |  | TT |  | AT=K9TCC\_Ab |
| SCOT\_055 | ATb | TT |  |  |
| SCOT\_610 | AT | TT |  |  |
| SCOT\_364 | AT | TT |  |  |
| SCOT\_858 | AT | TT |  |  |
| SCOT\_148 | ATc |  |  |  |
| SCOT\_223 | AT | TT |  |  |
| SCOT\_477 | AT | TT |  |  |
| SCOT\_374 |  |  |  | AT=K9TCC\_An |
| SCOT\_408 | TTa | TT |  |  |
| SCOT\_463 |  | TT | AT |  |
| SCOT\_970 | AT | TT |  |  |
| SSHP\_319 | AT | TT |  |  |
| SSHP\_160 | AT | TT |  |  |
| SSHP\_530 | AT | TT |  |  |
| SSHP\_588 | AT |  |  |  |
| SSHP\_750 | AT | TT |  |  |
| SSHP\_789 | AT | TT |  |  |
| SSHP\_970 | AT | TT |  |  |
| SHIH\_488 | ATc |  |  |  |
| WEST\_517 | AT | TT |  |  |
| WEST\_200 | ATa | TT |  |  |
| WEST\_956 |  |  | AT |  |
| WEST\_309 | TTa |  | TTa |  |
|  |  |  |  |  |
| a) Genotype confirmed by next-gen sequencing | | | |  |
| b) Genotype from next-gen sequencing, not detected by other method(s) | | | | |
| c) Genotyped by Sanger sequencing from cDNA | | | |  |
| d) Sample match of urine and tumor was inconclusive due to poor sample condition | | | | |

**Supplemental Table 3.** Clinical Characteristics of Dogs With and Without the Mutant BRAF(V595E). Dogs providing samples for this study included 61 dogs with naturally-occurring invasive transitional cell carcinoma who were undergoing evaluation and treatment at the Purdue University Veterinary Teaching Hospital, and 5 dogs undergoing evaluation at other veterinary hospitals. Samples were collected and information gathered from medical records following informed pet owner consent and with the approval of the Institutional Animal Care and Use Committees.

|  |  |  |
| --- | --- | --- |
| Characteristica | Dogs With the Mutation  n=58a | Dogs Without the Mutation n=8a |
| Age at Diagnosis, Years  Based on 53 dogs with mutation and 8 dogs without mutation.a | Median 10  Range 7-16 | Median 12  Range 5-18 |
| Gender, Number of Dogs (%)  Based on 53 dogs with mutation and 8 dogs without mutation.a | Spayed Female 31 (58%)  Intact Female 0 (0%)  Neutered Male 21 (40%)  Intact Male 1 (2%) | Spayed Female 6 (75%)  Intact Female 0 (0%)  Neutered Male 1 (12.5%)  Intact Male 1 (12.5%) |
| Breeds, Number of Dogs | 13 Mixed Breed  32 High-Risk Breedb  13 Other Breedsc | 4 Mixed Breed  2 High-Risk Breedb  2 Other Breedsc |
| TNM Stage for Canine Bladder Cancerd at Diagnosis, Number of Dogs (%)  Based on available information on 61 dogs.a    T2d (Invasion Into Bladder Wall)  T3d (Spread Through Bladder Wall or to Organs Adjacent to Bladder and Urethra)  N0 (No Detected Node Involvement)  N1 (Nodal Involvement Detected)  M0 (No Detected Distant Metastasis)  M1 (Distant Metastases Detected)  Metastases in Any Site At Diagnosis  (nodal and/or distant) | 41 (77%)  12 (33%)  51 (96%)  2 (4%)  49 (92%)  4 (8%)  5 (9%) | 8 (100%)  0 (0%)  8 (100%)  0 (0%)  8 (100%)  0 (0%)  0 (0%) |
| TNM Stage at Death, Number of Dogs (% of Dogs with Information Available)  TNM Stage at Death Assessabled  TNM Stage at Death Not Known  Dogs Still Alive  T2 (Invasion Into Bladder Wall)  T3 (Spread Through Bladder Wall or to Organs Adjacent to Bladder and Urethra)  N0 (No Detected Nodal Involvement)  N1 (Nodal Involvement Detected)  M0 (No Detected Distant Metastasis)  M1 (Distant Metastases Detected)  Metastases At Any Site at Death | 40 (75%)  12 (23%)  1 (2%)  29 of 40 dogs (72.5%)  11 of 40 dogs (27.5%)  25 of 40 dogs (62.5%)  15 of 40 dogs (37.5%)  15 of 40 dogs (37.5%)  25 of 40 dogs (62.5%)  27 of 40 dogs (67%) | 5 (62.5%)  2 (25%)  1 (12.5%)  5 of 5 dogs (100%)  0 of 5 dogs (0%)  4 of 5 dogs (80%)  1 of 5 dogs (20%)  2 of 5 dogs (40%)  3 of 5 dogs (60%)  3 of 5 dogs (60%) |
| Medical Treatment Givene, Number of Dogs (%). Based on information on 61 dogs.a  No Treatment Given After Diagnosis  Chemotherapy Givene  Cyclooxygenase Inhibitor Was Only  Treatment Givene | 2 (4%)  43 (81%)  8 (15%) | 0 (0%)  6 (75%)  2 (25%) |
| Response to Medical Treatmenta,f  Number of Dogs (% of Those Treated)  Partial Remission  Stable Disease  Progressive Disease  Response Not Known | 10 (21%)  32 (63%)  6 (12%)  3 (6%) | 1 (12.5%)  3 (37.5%)  2 (25%)  2 (25%) |
| Survival From Diagnosis to Death (Days)a | Median 265  Range 0-797 | Median 197  Range 0-772 |

a) Information regarding age, gender, tumor stage, and treatment response was available for 53 dogs with the mutant BRAF and 8 dogs with wildtype BRAF. Information on the other 5 dogs was limited to the breed of the dog.

b)Breeds of dogs reported to have significantly higher risk of TCC compared to mixed breed dogs (Knapp, et al., 2014) included: Scottish Terrier, West Highland White Terrier, Beagle, Shetland Sheepdog, Wirehair Fox Terrier.

c) Other breeds included: German Shepherd Dog, Australian Shepherd, German Shorthair Pointer, Labrador Retriever, Miniature Dachshund, Miniature Pinscher, Shih Tzu, Jack Russell Terrier, Miniature Schnauzer

d)The TNM staging system for canine bladder cancer (Owen, 1980) differs from the staging criteria for human bladder cancer with muscle invasive tumors being classified as T2 in dogs and T3 or above in in most staging systems in humans. T3 tumors in dogs are those that have spread to perivesicular structures beyond the bladder and urethra (prostate, vagina, etc.).

e)Medical therapy is the main form of cancer treatment given to dogs with bladder cancer (Knapp, et al. 2013). In addition to chemotherapy, cyclooxygenase inhibiting drugs have antitumor activity in dogs with TCC (Knapp, et al. 2013; Knapp, et al. 2014). The drugs given to dogs with the mutation included: vinblastine, carboplatin, mitoxantrone, cyclophosphamide, 5-azacitidine, chlorambucil, mitomycin C, a folate-vinblastine conjugate, piroxicam, deracoxib, and firocoxib. The drugs given to dogs without the mutation included: mitoxantrone, vinblastine, cyclophosphamide, chlorambucil, a folate-tubulysin conjugate, zebularine, deracoxib, and piroxicam.

f) Tumor responses were defined as follows: partial remission (≥50% reduction in tumor volume and no new tumor lesions), stable disease (<50% change in tumor volume and no new lesions), and progressive disease (≥50% increase in tumor volume of the development of new tumor lesions). Complete remission did not occur in any of the dogs reported here.

References:

Knapp DW, McMillan SK. 2013. Tumors of the urinary system. In: Withrow SJ, Vail DM, eds. Withrow and MacEwen’s Small Animal Clinical Oncology, 5th ed. St. Louis: Elsevier – Saunders. p 572-582.

Knapp DW, Ramos-Vara JA, Moore GE, Dhawan D, Bonney PL, Young KE. Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. ILAR J, In press, 2014.

Owen LN. 1980. TNM classification of tumours in domestic animals, Geneva, World Health Organization.