Mutation Landscape and Clinical Actionability in Canine Melanoma

Esther Chon[1], Jonathan Adkins[1], Natalie Duran[1], Jessica Molnar[1], Manisha Warrier[1], Salvatore Facista[1], Shukmei Wong[1], Nidhi Patel[1], Derick Whitley[1], Kathryn Banovich[1], Zhanyang Zhu[1], Martin Boateng[1], David Haworth[1], Sharadha Sakthikumar[1], Guannan Wang[1], William PD Hendricks[1]

[1]The Translational Genomics Research Institute (TGen) and Vidium Animal Health, a Subsidiary of TGen

INTRODUCTION

Malignant melanomas account for most canine oral malignancies and also occur at other primary sites[1]. Despite aggressive conventional therapies, most dogs, especially with oral malignant melanoma, eventually succumb to this disease[2]. A rapidly growing genomic understanding of canine melanoma (Figure 1) alongside an increasing arsenal of genomically-guided therapeutic options for human cancers is now pointing to new potential therapeutic targets for canine melanoma. To uncover actionability horizons, we aimed to evaluate the genomic landscape of canine malignant melanoma and identify biomarkers with diagnostic, prognostic, and therapeutic associations using a canine targeted sequencing genomic panel, SearchLight DNA®.

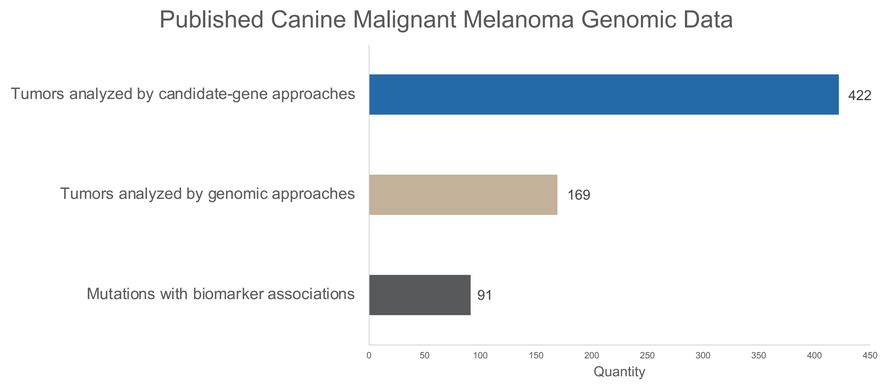

Figure 1. Published canine malignant melanoma genomic data. Primary canine publications were reviewed to quantify individual malignant melanoma cases that were analyzed either by candidate-gene approaches (such as PCR or Sanger sequencing) or by genomic approaches (such as whole genome sequencing or targeted sequencing). Mutations bearing diagnostic, prognostic, and/or therapeutic biomarker associations were also quantified.

METHODS

Malignant melanoma samples were profiled with SearchLight DNA®, a canine cancer gene sequencing panel. SearchLight DNA® utilizes next-generation sequencing to identify multiple mutation types, including single nucleotide variants (SNVs), copy number variants (CNVs), and internal tandem duplications (ITDs) in 120 relevant cancer genes (Figure 2). Mutations are then annotated with diagnostic, prognostic, and predictive biomarker associations from published, peer-reviewed literature curated in Vidium Insight™, Vidium's knowledge database (Figure 3). Diagnostic biomarkers are mutations found to be enriched in a tumor type, are predicted to be pathogenic, and/or contributes to cancer development (i.e. functional data supports their role in cancer). Prognostic biomarkers are mutations that are associated with outcome or drug response. Therapeutic biomarkers are mutations associated with response to therapy. Evidence levels supporting these biomarkers are categorized based on consensus guidelines from the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and the College of American Pathologists (CAP, Figure 4)[3]. Mutations with biomarker associations, and patients whose tumors bore those mutations, were identified by SearchLight DNA® and quantified. FDA-approved targeted therapies for which evidence supports a mutation-based sensitivity association were also identified.

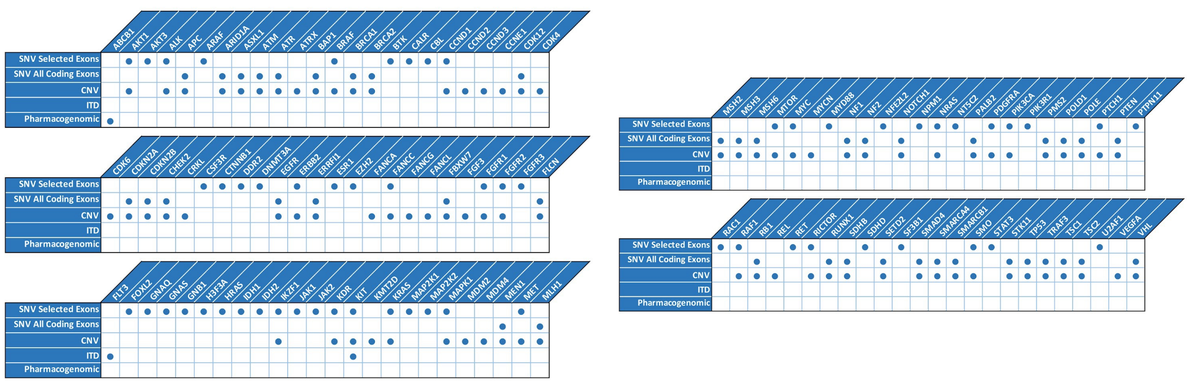

Figure 2. Genes and variant types evaluated by SearchLight DNA®. SearchLight DNA® is a tumor-only, next-generation sequencing, hybrid-capture, canine gene panel covering 120 genes associated with canine or human cancer. Mutation types identified include single nucleotide variants (SNV), copy number variants (CNV), and internal tandem duplications (ITD).

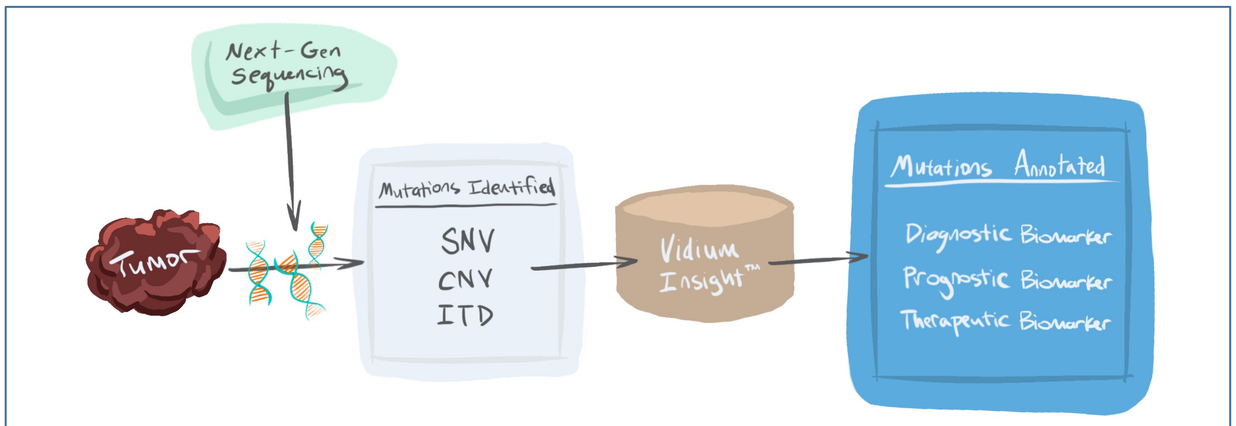

Figure 3. Mutation identification and annotation workflow of SearchLight DNA®. Next generation sequencing is performed on a submitted tumor to identify mutations (SNVs, CNVs, and ITDs) in 120 genes associated with canine or human cancer. These mutations then go through Vidium's bioinformatics pipeline to get annotated as a biomarker of diagnosis, prognosis, and/or therapy based on published literature curated and stored within Vidium's knowledgebase, Vidium Insight™. Annotation of these mutations provides meaning to each mutation for clinical application by the ordering clinician.

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence

FDA-approved therapy Included in professional guidelines

Level B Evidence

Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence

FDA-approved therapies for different tumor types or investigational therapies

Level D Evidence

Preclinical trials or a few case reports without consensus

Tier III: Variants of Unknown Clinical Significance

Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases

No convincing published evidence of cancer association

Tier IV: Benign or Likely Benign Variants

Observed at significant allele frequency in the general or specific subpopulation databases

No existing published evidence of cancer association

Figure 4. Evidence levels supporting biomarker associations for mutations identified by SearchLight DNA®. Supporting evidence levels for every diagnostic, prognostic, and therapeutic association with identified mutations are guided by consensus recommendations of the AMP, ASCO, and CAP that are robustly used within the human genomic space.

RESULTS

Confirmed malignant melanomas were identified in 66 dogs, 55 of which were from the oral cavity, with 8 from other primary sites. The majority of cases were identified to have mutations carrying diagnostic, prognostic, and/or therapeutic associations (Figure 5). Six-hundred and seventy-four mutations were identified in 84 genes, with TP53 being the most frequently mutated gene followed by MDM2 and CDK4. Sixteen genes bore mutations with evidence supporting sensitivity to FDA-approved targeted therapies, accounting for 51 patients for whom therapeutic biomarkers were associated with 15 FDA-approved targeted therapies (Figure 6). Four of these drugs are readily available to veterinarians from at least 1 major compounding pharmacy.

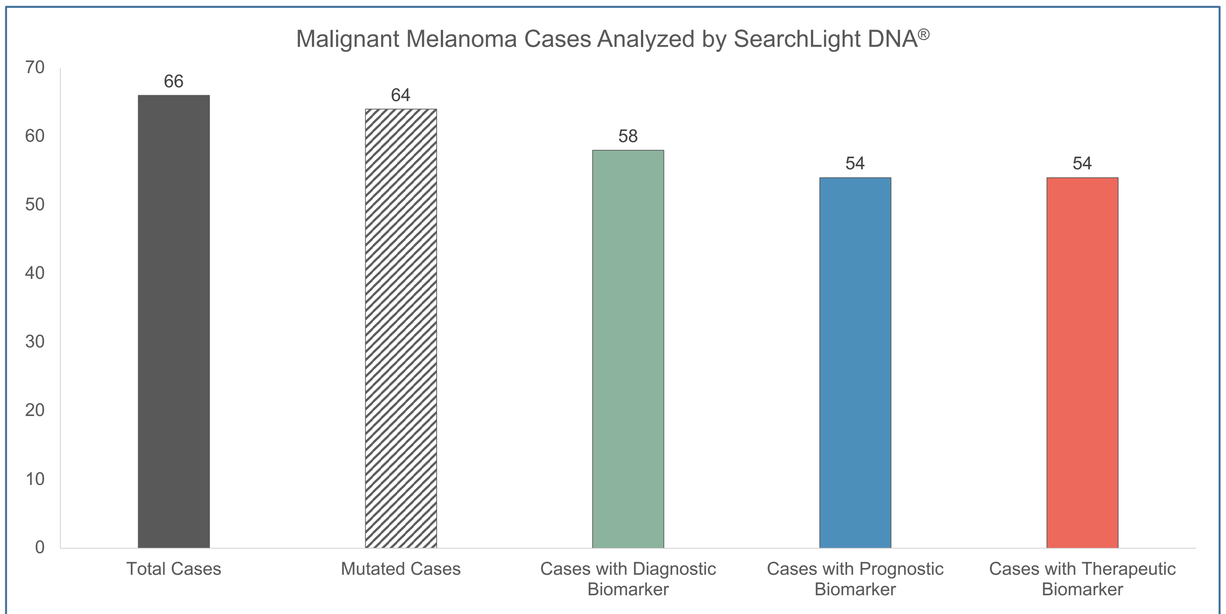

Figure 5. Melanoma cases analyzed by SearchLight DNA®. Of 66 melanoma cases, mutations were identified in ~97% of cases. Identified mutations were associated with diagnosis, prognosis, and therapy in 58, 54, and 54 cases, respectively, highlighting the broad clinical utility of SearchLight DNA® for canine malignant melanoma.

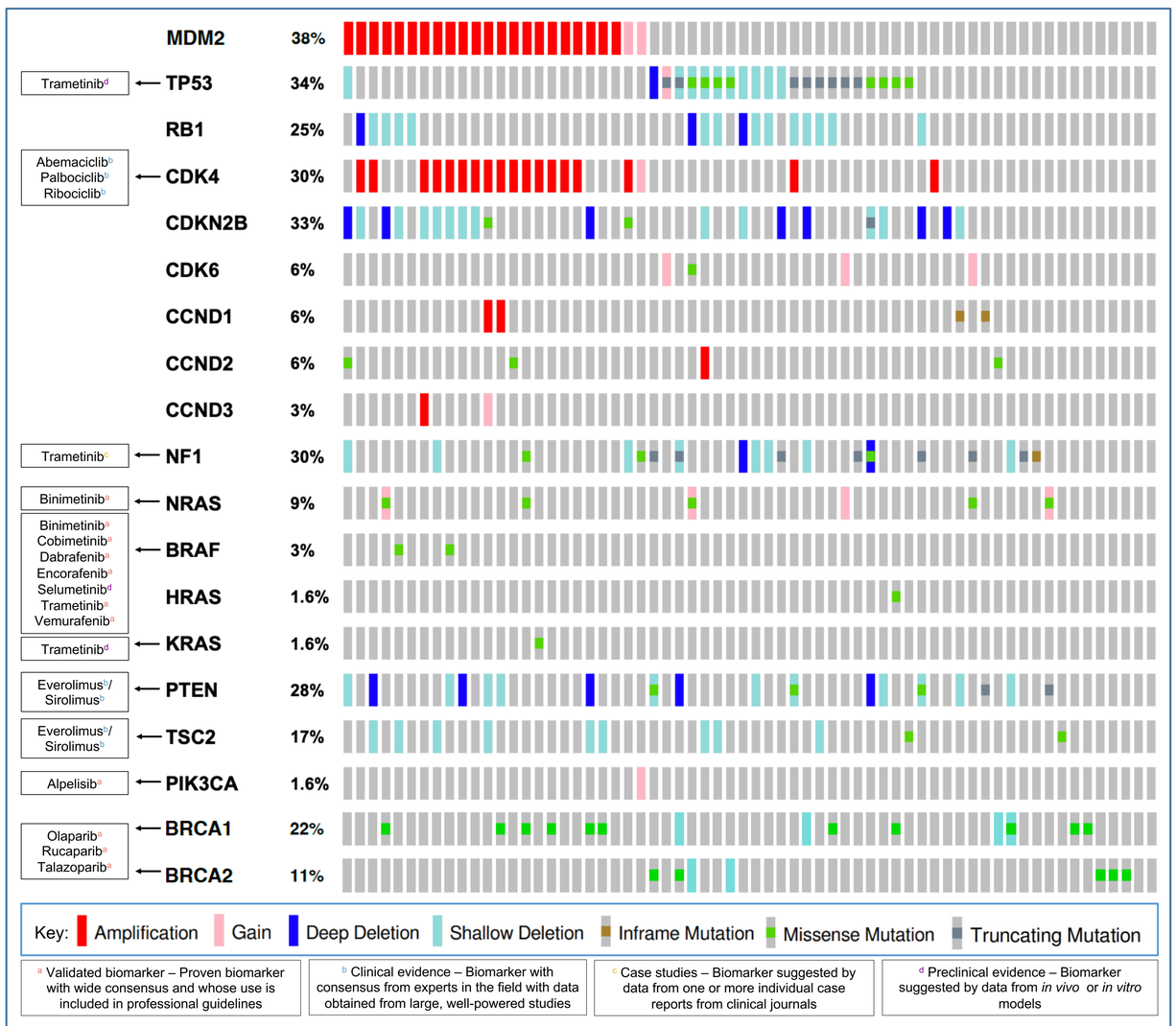

Figure 6. Oncoprint of mutations in melanomas analyzed by SearchLight DNA®. Genes identified to be mutated in canine malignant melanoma cases are broadly grouped according to signaling pathways. Genes with mutations annotated as therapeutic biomarkers point to their respective FDA-approved targeted therapies to the left. This is not an exhaustive list of the mutations identified within the melanoma cohort analyzed by SearchLight DNA®.

CONCLUSIONS

- Understanding the genomic landscape of canine malignant melanoma in the context of mutation-based biomarker evidence supports the use of targeted therapies already available to veterinarians.
- Transparency of evidence supporting all biomarker associations is necessary, especially therapeutic associations that often infer from the human genomic space.
- In the absence of primary data demonstrating efficacy of targeted therapies in canines, evidence levels to support therapeutic associations should be as high as possible when inferring between species (humans to canines) and across different cancer types.
- These data present opportunities to leverage human FDA-approved therapies in veterinary patients.

FUNDING & ACKNOWLEDGEMENTS

We thank all veterinarians and pet parents who pursued SearchLight DNA for genomic analysis. *Financial disclosures: This study was funded by Vidium Animal Health (VAH), a TGen subsidiary. All authors are employees of TGen and Vidium Animal Health.*

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