# Precision Medicine in Veterinary Science

Esther Chon, DVM[a], William Hendricks, PhD[a], Michelle White, DVM, PhD[b], Lucas Rodrigues, DVM, PhD[b], David Haworth, DVM, PhD[a], Gerald Post, DVM, MEM[b,*]

**KEYWORDS**

• Targeted therapy • Genomics • Personalized medicine • Individualized medicine • Genomic panels • Dogs • Canine • Cancer

**KEY POINTS**

- The success of precision medicine for humans is based on the understanding of cancer's great genomic heterogeneity and the successful pairing of therapies with the genomic mutations they target.
- Genomic changes shared between human and canine tumors have been the basis for predictions that human-approved drugs can be successfully used in canine oncology, and these hypotheses have shown early initial promise.
- Multiple genomic assays, spanning early cancer detection to treatment and monitoring, are currently available to all veterinarians.
- A growing body of information is available surrounding the safety and early efficacy of targeted therapeutics in dogs.

## BACKGROUND

### *Human Precision Medicine*

***The human cancer precision medicine paradigm has been built on the foundation of understanding cancer's genomic underpinnings and great individual variability***

Remarkable improvements in human cancer patient outcomes have been achieved in recent decades, progress best represented by the dramatic drop in cancer death rates. As of 2020, death rates have dropped by 33% relative to their peak in 1991, accounting for 3.8 million lives saved. This progress is due to improvements in treatment, early detection and diagnosis, and management of risk factors, all of which are components of the precision medicine paradigm arising over the past 30 years.[1] "Precision medicine" is defined by the National Institutes of Health's National Cancer Institute (NCI) as: "A form of medicine that uses information about a person's own genes or

[a] Vidium Animal Health, 7201 East Henkel Way, Suite 210, Scottsdale, AZ 85255, USA; [b] OneHealthCompany, Inc, 530 Lytton Avenue, 2nd Floor, Palo Alto, CA 94301, USA
* Corresponding author.
*E-mail address:* gerry@fidocure.com

Vet Clin Small Anim ■ (2023) ■–■
https://doi.org/10.1016/j.cvsm.2023.12.006 **vetsmall.theclinics.com**

proteins to prevent, diagnose, or treat disease. In cancer, precision medicine uses specific information about a person's tumor to help make a diagnosis, plan treatment, find out how well treatment is working, or make a prognosis…".[2] A common misconception of cancer precision medicine dispelled by this definition is that precision medicine refers solely to the use of cancer gene sequencing tests to guide the selection of targeted treatments. Although this type of testing (cancer gene sequencing) and this use case for the testing (treatment guidance) are important examples of clinical tools and scenarios at the forefront of precision care delivery culminating from decades of research, they are only part of many aspects of the precision medicine approach. Critically important broader aspects of precision medicine in the NCI's definition include: (1) broader emphasis on uses of individual patient molecular information (whether genes, proteins, or other factors) to supplement what has historically been a more "one-size-fits-all" model focused on phenotypes (clinical signs, symptoms, imaging, and pathology), and (2) broader emphasis on not just use of molecular information for drug selection but also for guiding prevention, diagnosis, and prognostication.

The precision medicine paradigm not only includes clinical practice as emphasized in the NCI's definition but also translational research where it guides an improved understanding of tumor biology, tumor classification, and development of new drugs and diagnostics—all in the setting of individual patient variability and a molecular understanding of cancer in individual patients. The human cancer precision medicine paradigm has been built on the foundational recognition of cancer's genetic basis and our growing understanding of its vast and often subtly hidden individual variability.[3–5] Through a series of studies in human colorectal cancer more than 30 years ago that tracked the genetic progression of these tumors from adenomas to advanced metastatic disease, it became clear that cancer occurs when mutations in genes that regulate cell life, cell death, and cell:cell interactions accumulate clonally in expanding cell populations.[5] At the convergence of genetics, cell and functional biology, and clinical research, it then became clear that these cancer gene mutations give rise to aggressive cell- and tissue-level phenotypes, such as excessive growth or invasion, leading to the formation of malignant tumors that spread through tissues, organ systems, and entire organisms.[3] The background genetics and environment of the whole organism as well as the individual initiating cell in which these mutations arise can also alter the trajectory of developing cancers in different patients even though we often clinically observe relatively high levels of phenotypic convergence based on clinical presentation, imaging, and histology within individual tumor types.[4] The vast potential genomic variability hiding below the surface of any individual cancer diagnosis was first made clear in the mid-2000s after a revolution in DNA sequencing technology drove the cost and turnaround time of sequencing a single genome down from greater than $100M in years in 2000 to less than $1k and 24 hours today.[6] This allowed us to move from evaluating individual mutations in a handful of patients in the 1990s to characterizing entire genomes in thousands of patients today alongside deep analysis of many other molecules (eg, RNA, protein, epigenomic marks) and functional studies in tandem. These studies have reshaped our understanding of cancer by uncovering the great genomic complexity below the surface of any given tumor type.[7]

### *Milestones in the emergence of the human cancer precision medicine paradigm*

Across the decades-long complex history of the rise of human cancer precision medicine, several milestones capture the major shifts in clinical practice and the research discoveries that drove them. First, emerging from genetic studies of whole chromosomes in the 1960s and 1970s was the discovery that some mutations were highly specific for individual cancer types (ie, diagnostic). These mutations also, by virtue

of being necessary for both the initiation and ongoing survival of cancer cells, could potentially serve as drug targets unique to the cancer cells and thereby result in broad therapeutic windows and high response rates, in contrast to nonspecific effects of cytotoxic chemotherapy. The prototypical example is the discovery of the Philadelphia chromosome in chronic myelogenous leukemia (CML). This "chromosome" is a DNA translocation in which two separate chromosomes, 9 and 22, have broken and reassembled to create a new fusion gene, *BCR-ABL1*. The resulting protein is a constitutively active tyrosine kinase that drives excessive, malignant growth signaling. The *BCR-ABL1* translocation is highly sensitive and specific for CML diagnosis, occurring in most CML cases, and is regularly evaluated as part of the diagnostic workup. Drug studies culminated in the discovery in the mid-90s that the targeted small molecule tyrosine kinase inhibitor (TKI) imatinib (Gleevec) could selectively kill CML cells. Imatinib has since proven highly successful in treating CML patients.[8] These discoveries cemented the early recognition of the value of genetics in cancer diagnostics while also launching the targeted therapy paradigm that is a cornerstone of cancer precision medicine and, through many similar discoveries across many tumor types, has been enabling tailored cancer treatment based on the individual and not solely on histology.

The success of imatinib in CML brought hope at the turn of the millennium that new, tumor-type-specific drugs could be developed to exploit common vulnerabilities within certain histologies. This stimulated biology- and pathway-driven drug development programs associated with specific tumor types. However, cancer's genomic heterogeneity, even within tumor types, and its importance for drug design and treatment response were not yet fully appreciated until a second set of discoveries in the 2000s. These studies uncovered drug responses correlating with specific mutations that unlike *BCR-ABL1* in CML only occur in a subset of patients. Representative of these efforts were the parallel, but unique development paths for the targeted therapies gefitinib (Iressa, first-in-class epidermal growth factor receptor [EGFR] inhibitor) and vemurafenib (Zelboraf, first-in-class BRAF inhibitor). Gefitinib is a small molecule EGFR inhibitor that was originally developed agnostic to genetics and based instead on the recognition that EGFR is overexpressed in many human epithelial cancers including lung cancer. It was initially evaluated in unselected patient populations for patients with advanced non-small cell lung cancer (NSCLC). After receiving accelerated approval in 2003, it subsequently failed to show improved outcomes in confirmatory trials. Thus, AstraZeneca, the drug's manufacturer, agreed to withdraw the drug from the United States market. Meanwhile, genetic analyses in 2004 determined that constitutively activating *EGFR* mutations were present in $\sim$ 15% of Caucasian and 50% of Asian patients. This discovery led to new clinical trials that incorporated *EGFR*-mutant–patient subgroup analysis or stratification and the subsequent major finding of a greater than 50% response rate in *EGFR*-mutant patients alongside substantially longer progression-free survival (PFS) versus chemotherapy in the frontline setting. In 2015, gefitinib was then approved by the Food and Drug Administration (FDA) for the initial treatment of *EGFR*-mutant metastatic NSCLC alongside a companion diagnostic test for *EGFR* mutations.[9] Another example, in contrast to the *post hoc* rescue of gefitinib through genomic stratification, is that of vemurafenib, which was strategically developed in response to the 2002 discovery of activating *BRAF* mutations in $\sim$50% of metastatic cutaneous melanoma patients. Vemurafenib was the optimized result of a structure-guided drug design and discovery program aimed at disrupting mutated *BRAF*. Unlike gefitinib, it was specifically tested in *BRAF*-mutant melanoma patients where it was found to have a $\sim$50% response rate and to confer dramatic improvements in progression-free and overall survival relative to chemotherapy.[10] Since the early days of targeted and stratified drug development beginning

with imatinib in 2001, more than 100 molecularly targeted anticancer agents have been approved by the FDA, many alongside companion diagnostics (including gene sequencing panels) for use in select patient populations within and even across tumor types.[11,12] This stratification has cemented the utility of thinking about cancer as a genetic disease that should be treated not just by tumor type but also individual genomic subtype.

The final milestone in the initial development of the human cancer precision medicine paradigm was the discovery of cancer's massive heterogeneity. As genomic sequencing technology became more accessible in the late 2000s, a growing number of individual research teams as well as multisite genomic consortia developed for the express purpose of harmonized genomic characterization of large cancer populations began to map the genomic landscapes of cancer ultimately across tens of thousands of patients with cancer.[7,13,14] Discoveries emerging from these studies revealed that cancer was far more complex than even early genomic studies had suggested, with most tumors bearing at least several driver mutations alongside dozens, hundreds, and, in some cases, even thousands of passenger mutations. Overall, more than 295,439 unique, likely pathogenic mutations in 707 cancer genes have been identified in more than 200 cancer types from hundreds of thousands of human cancer cases (COSMIC v95).[15] Although broad mutation patterns often track by tumor type, a large potential number of permutations of these mutations mean that most individual cancers bear a unique genomic signature. Yet, these mutations do often converge on shared pathways that intersect the large and growing list of targeted therapies. These mutations are increasingly well understood and many are associated with significant clinical value. More than 5000 mutation-based biomarkers are used in diagnosis, prognostication and/or therapy guidance in human cancer with ~1500 such mutations included in FDA or National Comprehensive Cancer Network clinical guidelines.[16–18] Thus, this understanding of cancer's substantial genomic variability along with recognition of the biomarker value of mutations and the potential for targeted therapies to improve outcomes in the setting of particular mutations together have established the precision medicine paradigm in which a cancer's genomic makeup must be considered in the care of the individual patient.

***Human cancer precision medicine is improving patients' lives in routine practice today***

The above discoveries reflect broader trends in human oncology research and clinical practice that have refined our understanding of cancer's initiation and progression while also shaping the development of new cancer treatments. At the same time, they have shaped the emergence of a thriving new discipline and diagnostic workstreams in human cancer medicine. Precision medicine is now an established tool that is a fundamental component not only of cancer research but also of daily, routine, clinical practice. For example, molecular pathology has rapidly grown over the past 25 years to focus on the incorporation of genomic and molecular information into clinical practice. In the United States, there are more than 231 boarded molecular pathologists, 1240 medical geneticists, and 2600 members of the American Association of Molecular Pathology.[19] In addition, more than 80 cancer genetics or genomics laboratories exist in the United States across academia and industry, offering more than 1500 cancer genetic or genomic assays that are used in clinical practice and approved by regulatory agencies.

Although there is a much wider world of cancer genomics research, drug development, and diagnostics that comprises cancer precision medicine as discussed earlier, the term "cancer precision medicine" is often associated specifically with the use of cancer gene sequencing panels that evaluate many genes and mutations at once,

often in a pan-cancer setting and are regularly used to inform the management of human cancer patients (with many variations on when and how they are used). Questions about the value of such testing often center on cost, efficacy ("matching" and response rates), off-label drug use, and, in general, evidence to support the nonstandard decision-making that such tests can enable. Thus, many studies have sought to assess the clinical benefit of broad genomic profiling, typically for treatment selection in prospective clinical trials and often in pan-cancer settings, with well-known examples including the BATTLE, IMPACT, SHIVA, I-PREDICT, PERMED, and NCI MATCH studies.[20–24] Most of these studies show clear clinical benefit of some type, particularly in comparison to cytotoxic chemotherapy regimens. However, biomarker:drug match rates and response rates are sometimes low. These studies are often complex, nuanced, and challenging to interpret, particularly in regard to their real-world clinical value because they often focus on use in heavily pretreated cancer patients at academic centers and because, unlike in veterinary oncology, a robust standard of care exists for most human cancer patients and these patients also often have access to numerous clinical trials. However, evaluation of genomic diagnostic panels in real-world community hospital settings (ie, private hospital systems not affiliated with universities) has also been shown that they improve outcomes while reducing treatment costs and improving quality of life.[25,26] Meanwhile, a significant ongoing need exists for identification of new genomic biomarkers, development of new effective therapeutics alongside companion diagnostics to facilitate their use in high-impact settings, and continuing refinement of incorporation of genomic diagnostics into the clinical care stream.

***Summary***

Genomics and precision medicine are not only driving the leading edge of care but are also inextricably woven into the standard of care for most human cancer patients today. Advances in the genomic understanding of human cancer have been steadily bringing new and powerful tools to the human cancer clinic since the 1970s including new diagnostics (single cancer genes/mutations, cancer gene panels, and companion diagnostics) and new drugs, many of which are now developed in a genomically stratified setting. In veterinary oncology, although limited by dramatically fewer resources, we are increasingly equipped to bring this innovation to the care of pets with cancer both via inference and lessons learned from human cancer precision medicine as well as through growing progress being made directly in canine cancer research.

## *Veterinary Precision Medicine*

Veterinarians have a long history of using precision medicine for purebred dogs using breed as a proxy for certain clinically relevant genotypes in many situations including interpretation of diagnostics (eg, hematocrit in greyhounds[27]), recommendations for screening (eg, presurgical coagulation screening in Doberman Pinschers[28]), and drug choices (eg, caution with ivermectin in collies[29]). A major breakthrough for veterinary precision medicine was made possible with the publication of the first canine reference genome in 2005 by Lindblad-Toh and colleagues. This resource represents a significant milestone in veterinary and comparative medicine,[30] enabling the identification and characterization of canine genomic alterations, including disease-associated mutations, structural variants, and regulatory elements. Among these subsequent discoveries have been cancer-associated genomic variants and molecular markers.[31–35]

These studies have shed light on the underlying genomic alterations and molecular pathways that drive canine cancers, providing valuable insights into tumor

development and progression. By identifying specific genomic mutations, chromosomal abnormalities, and gene expression patterns particular to specific cancers, researchers have begun to characterize molecular signatures for some canine tumors.[36,37] Genomic and molecular knowledge has paved the way for developing diagnostic tools and targeted therapies.[38–42] Although the first version of the feline reference genome was published 1 year after its canine counterpart,[43] this draft was low coverage (2X) and highly fragmented. Relative to the dog genome, slow progress in the improvement and annotation of the feline genome from 2006 to 2020[44] has further delayed the potential applications of cancer genomics for this species.[45]

In dogs, the genetic diversity that exists across the entire genome is comparable to that across the human genome and thus expectations that certain targeted therapies may work in specific subpopulations of patients even in cases of poor overall drug efficacy in clinical trials is as valid in canine patients as it is for humans. Indeed, there may be greater hope for such successes in canine subpopulations because of the unique population structure of domestic dogs, with the overall diversity being siloed within breeds[46,47] and thus, even in mixed breed dogs, inherited according to recent breed ancestry. In other words, there is good reason to expect that a drug showing efficacy in only a small percentage of dogs in a genetically diverse canine clinical trial cohort or even no efficacy in a genetically homogenous canine clinical trial (eg, a single breed group) may prove extremely efficacious in a population enriched for a certain biomarker or shared ancestry.

### *Precision medicine and genetic tests for dogs with cancer*

Advances in genomic characterization of tumors are crucial for the implementation of precision medicine approaches to treat cancer in dogs. The molecular phenotyping of canine tumors has revealed striking similarities to those characteristics identified in humans, enabling translation of knowledge and therapeutic strategies from human medicine to veterinary oncology. As a result, precision treatments that have proven effective in human patients are now being adapted and used in dogs, enhancing their chances of successful treatment outcomes,[40] advancing our understanding of cancer biology, improving treatment options for both species,[48] and offering the potential to streamline cancer drug development pipelines.[49] However, significant challenges still arise from the limited genomic information available for certain canine tumor types, limiting the possibility of comparative precision medicine trials. Representative genetic profiles of cancer types such as thyroid carcinoma, anal sac adenocarcinoma, neuroendocrine tumors, and others have been published only recently,[35] and many more studies of various cancer types are needed.

As genomic research is completed, the implementation of precision medicine can be accelerated in veterinary medicine compared with human medicine because of the relative paucity of regulations in veterinary medicine.[50,51] This increased flexibility in veterinary medicine increases the number of options available for pets in terms of treatment, including deviations from typical “first-line” protocols, off-label drug uses, and drug combinations.

Technological advancements facilitated the development of specific diagnostic tests, such as the identification of the V595E mutation of the *BRAF* gene in DNA found in the urine of dogs with urothelial carcinoma or transitional cell carcinoma. *BRAF* V595E has been identified in 75% to 80% of dogs with urothelial carcinoma[38,39] and can be identified in urine and bladder biopsies in the early stage of disease and thus allow faster accurate diagnosis of affected dogs.[52]

Continued advancements have led to development of several next-generation sequencing (NGS) assays in veterinary medicine,[53–55] two of which are currently

commercially available. These genomic tests can be performed using DNA from formalin-fixed paraffin-embedded (FFPE) tissue or fine-needle aspirates (FNAs) facilitating their incorporation into clinical routines. In 2019 and 2020, the One Health Company and Vidium Animal Health launched FidoCure and SearchLight DNA, respectively. Both assays, available for the veterinary community, harness the power of NGS platforms to create comprehensive genomic profiles of canine cancers, facilitating the identification of mutated oncogenes and tumor suppressor genes in canine cancer and enabling genomic-guided small-molecule targeted therapy.

Recently, circulating cell-free DNA (cfDNA) has been used for cancer diagnosis in dogs. cfDNA refers to small fragments of DNA that circulate in the plasma. These fragments are released during the turnover of apoptotic and necrotic cells, including both normal and cancer cells.[56] cfDNA is typically found at lower levels in healthy patients and higher levels in patients with cancer. In veterinary medicine, various approaches involving cfDNA have been investigated. For instance, researchers have explored the measurement of cfDNA concentration to assess prognosis of dogs with cancer.[57–59] Small genomic alterations have been detected from DNA in plasma from dogs with cancer using polymerase chain reaction (PCR),[60] and NGS has enabled the identification of different types of genomic alterations that, in combination with bioinformatic algorithms, can detect cancer in dogs with an overall sensitivity across all cancer-diagnosed dogs of 54.7%.[61] The utility of cfDNA has been evaluated in different aspects of clinical oncology for different tumor types. Measurement of cfDNA associated with DNA integrity index using fragments of long interspersed nuclear element-1 is a valuable biomarker for disease progression monitoring in dogs with oral malignant melanoma.[62] Another non-genomic (nucleosome-based) liquid biopsy assay is also commercially available.[63] Liquid biopsy has provided valuable insights for not only cancer diagnosis but also therapy selection, treatment response, and disease monitoring.[64,65] Genomic characterization enables identification of specific genetic alterations that guide targeted therapy selection. It allows clinicians to tailor therapies to the unique genomic profile of the tumor, enhancing treatment efficacy and minimizing unnecessary side effects.[66,67]

### *Targeted therapy in dogs with cancer*

Several different types of targeted therapies are currently used in veterinary medicine, including small molecule inhibitors and monoclonal antibodies (mABs). Small molecule inhibitors cause a direct effect on tumor cells, competitively inhibiting receptors in a reversible or irreversible manner. A major category of small molecule inhibitors in veterinary medicine are those inhibiting tyrosine kinases (mediators of signaling pathways of cell proliferation, differentiation, migration, angiogenesis, and cell-cycle regulation). TKIs effectively hinder the kinase's ability to phosphorylate itself and initiate downstream signaling cascades.[68,69] To ensure selectivity for specific proteins, researchers have extensively characterized the adenosine triphosphate (ATP)-binding pockets of various kinases. This knowledge enables design of inhibitors exhibiting activity against restricted subsets of kinases, thereby minimizing off-target effects on non-targeted kinases. These inhibitors are often amenable to large-scale synthesis, possess oral bioavailability, and readily penetrate cells to reach their intended targets. Toceranib phosphate (Palladia, Zoetis) and masitinib (Masivet, AB Science) were the first TKIs approved by the FDA and the European Medicine Agency, respectively, for dogs with mast cell tumors (MCTs). These small molecules have potent inhibitory activity against members of the kinase receptor families such as VEGFR, PDGFR, RET, and Kit, resulting in antitumor and antiangiogenic effects.[70,71]

Small molecules can block specific pathways related to carcinogenesis, tumor growth and block specific enzymes, growth factors, and receptors responsible for cell proliferation.[72] Small molecules are commonly recommended for human cancer treatment, usually when a specific target is identified from NGS-based companion diagnostics that inform who could benefit from this specific treatment. This precision medicine approach has already been used to treat canine cancers.[40] Because there is an overlap of 50 well-known oncogene and tumor suppressor genes, including hotspot mutations between both species,[35] treatment using small molecules guided by genetic alterations are likely to also bring benefits for dogs with cancer.

Instead of having a direct effect on tumor cell activity, mABs are engineered to bind to specific proteins on the cancer cells and have unique immune-effector mechanisms, such as antibody-dependent cellular toxicity, complement-dependent cytotoxicity, and complement-dependent cell-mediated cytotoxicity.[73] Unfortunately, clinical trials assessing the efficacy of two caninized mABs developed by Aratana Pharmaceuticals for the treatment of B-cell and T-cell canine lymphoma, respectively, have yielded disappointing results, potentially because of nonspecific binding activities of these antibodies.[74] On the other hand, the immune checkpoint blocking programmed cell death 1 has been under investigation with promising results both in vitro and in vivo,[75–78] especially for melanomas,[79,80] and anal sac adenocarcinoma.[81]

The use of small molecules and mABs to treat cancer in dogs provides an opportunity for bespoke and targeted treatments that are tumor-specific rather than delivering systemic toxic chemotherapies to deplete all rapidly dividing cells. Because of the paucity of veterinary-approved small molecules available and the fact that veterinarians can legally use drugs approved for human use as long as there is no commercially available veterinary equivalent, human small molecule therapies have been used with the outcomes reported in the literature. For example, in canine transitional cell carcinoma, dysregulation in the EGFR signaling pathways is present in the majority of cases. As no veterinary-specific EGFR inhibitor is approved, the human-approved EGFR inhibitor, lapatinib, has been used off-label and shown efficacy in treating this disease.[41]

Precision medicine approaches have not just been used to improve companion animal care. Genomic data associated with proteomic, transcriptomic, and metabolomic data have been used as important tools in conservation medicine as they do not require species-specific diagnostic tests. Hypotheses can be examined and validated through computational approaches without being constrained by the availability of additional samples. Whilde and colleagues discussed several case studies where precision medicine was used to help with conditions affecting threatened species including fibropapillomatosis in sea turtles, tumors in beluga whales, Ebola virus in African great apes, chytridiomycosis in amphibians, and facial tumor disease in Tasmanian devils.[82]

Overall, precision medicine has the potential to revolutionize the way we diagnose and treat canine cancer. Although much more research is needed in this area, early results are promising, and it is likely that precision medicine will play an increasingly important role in veterinary oncology in the years to come.

## DISCUSSION

### *Guidelines*

***WHY would one consider precision medicine for the canine cancer patient?***

**The genomic homology between people and dogs lends support to the same success of precision medicine in dogs as it has in people.** The utilization of an individual's cancer genomic signature for the selection of specific targeted therapies has proven successful in human oncology, improving outcomes and quality of life for patients with cancer.[9–12,25,26] Shared molecular mechanisms between dogs and humans, along

with orthologous genomic alterations in cancer genes affecting corresponding biological pathways, support the potential benefit of using human genomic information for clinical inferences in dogs.[48,49] In fact, the structured analysis of sequence conservation and conversion of human mutations to the canine genome ("caninisation") has recently been applied to COSMIC, the most prominent human cancer mutation database, identifying shared putative cancer-driving mutations and mutations bearing similar biomarker associations with diagnostic, prognostic, and therapeutic utility.[83] This structured caninization of human cancer mutations facilitates the interpretation and annotation of canine mutations, allowing for the reasonable inference of mutation-based biomarker data from the information-rich human oncology space and responsibly meeting the clinical needs of canine cancer patients.

**Precision medicine is widely available for dogs, with utility in all steps of the cancer journey.** There is already emerging evidence of precision medicine's clinical benefit in dogs. By leveraging the caninization of the abundant human mutation-based biomarker information, genomic testing in dogs has demonstrated utility in providing diagnostic guidance, prognostic support, and therapeutic options for canine cancer patients, particularly those that have ambiguous diagnoses and therefore are inherently challenging to manage.[84] A recent real-world clinicogenomics study unveiled gene-level prognostic indications for several cancer genes and potential association of mutant genes with response to targeted therapies.[40] A separate study identified novel mutations with prognostic value and demonstrated the benefit of targeted therapies, particularly those that are genomically informed, across multiple cancer types in dogs.[85] This therapeutic utility of genomic analysis allows for more effective clinical decision-making for treatment interventions with targeted therapeutics that could eventually prove to have synergy with or even superiority over conventional therapies. Another meaningful avenue of genomics is screening for early cancer detection and for cancer monitoring. An NGS-based liquid biopsy technique has demonstrated utility as a novel option for noninvasive multi-cancer detection in dogs.[61,86,87] In their entirety, these bodies of work provide a compelling view of the significant potential in genomics and precision medicine for dogs with cancer. Resulting genomic and outcome data gathered from these genomic analyses could then feed back into a data pool that could ultimately guide novel drug development for dogs and people with cancer.

**Owing to the heterogeneity of cancer, there is a need for individualized testing.** The explosion of molecular technology has highlighted the inter- and intra-tumoral heterogeneity within cancer types as well as across different cancers in both dogs and people.[88–90] Appreciation of this genomic diversity calls for individualized testing using diagnostic assays to characterize a broad range of cancer types. For people, as more molecularly guided treatments become FDA-approved, companion diagnostics are developed alongside them to inform selection of patients for these targeted approaches. In veterinary medicine, fewer though still highly impactful assays are increasingly available and easily accessible, enabling our canine patients to shift away from the "one-size-fits-all" therapeutic paradigm to one that is more personalized and biomarker-guided.

### *WHAT genomic tests are currently available for dogs?*

**Multiple precision medicine tools are already commercially available and increasingly used in dogs.** For dogs with cancer, there are currently several genomic assays available. Two of these use NGS technology to simultaneously evaluate multiple mutation types in multiple genes across a variety of cancers. SearchLight DNA (Vidium Animal Health) identifies copy number variants, single-nucleotide variants, and internal

tandem duplications in 120 cancer genes. Mutations are then annotated as biomarkers of diagnosis, prognosis, and therapy, with supporting evidence levels from published literature for each biomarker association. Fidocure (The One Health Company) sequences the entire coding region of 56 commonly mutated cancer genes, identifying single-nucleotide variants, insertions and deletions, and copy number variants. Mutations identified in each patient and the relevant scientific evidence for each variant's relevance for prognosis and therapy guidance are described in a unique patient report, and therapies can be ordered and delivered to the patient's home through Fidocure's partner compounding pharmacies.

There are several other tests that are focused on evaluating one or two genes for specific cancers, using the PCR method. PARR (PCR for antigen receptor rearrangements; offered by multiple institutions and companies) evaluates clonality of T-cell receptor and/or immunoglobulin heavy chain genes to immunophenotype and/or distinguish lymphoproliferative neoplasia from inflammation. Other available tests include C-kit PCR (for internal tandem duplication mutation in exon 8 and/or exon 11 in the c-kit gene; Michigan State University [MSU]); PTPN11 mutation PCR (for E76K substitution mutation in the PTPN11 gene; MSU); transmissible venereal tumor (TVT) PCR (for long interspersed elements in the cellular myelocytomatosis oncogene [c-MYC] gene; MSU); CADET *BRAF* and CADET *BRAF-PLUS* (for V595E and copy number mutations in the BRAF gene; Antech) to aide in the diagnosis of MCT/gastrointestinal stromal tumor (GIST), melanoma, histiocytic sarcoma, TVT, and urothelial carcinoma, respectively.

For early cancer detection in dogs, OncoK9 (PetDx) is a genomic screening test that uses the liquid biopsy method. OncoK9 detects cfDNA—specifically a fraction of cfDNA called the circulating tumor DNA (ctDNA) that originates from tumor cells—via NGS. A clinical validation study demonstrated this assay's detection of cancer signal in patients representing 30 distinct cancer types.[61]

***WHEN should one consider precision medicine?***

**There are many clinical scenarios where precision medicine tools should be considered for dogs: diagnostic guidance, prognostication, therapeutic options, cancer screening, and cancer monitoring.** *Diagnosis*: Genomic tests that are already commonly used in the diagnostic setting are the PCR-based tests (CADET *BRAF*, PARR, and so forth). These tests aid in the diagnosis of specific cancers if they are highly suspected from first-line pathologic evaluation. SearchLight DNA, an NGS-based assay, can also be used for diagnostic clarification[84] in cases that are diagnostically ambiguous, based on annotation of identified mutations using human consensus guidelines.

*Prognosis*: Cancer prognostication can be performed with both SearchLight DNA and Fidocure. For SearchLight DNA, the same process that is used to annotate diagnostic and therapeutic biomarkers is also used to annotate identified mutations as prognostic. A recent study that used SearchLight DNA identified six genes that were associated with shorter PFS. This same study also revealed genomically informed targeted therapy given before first progression was associated with a significantly longer PFS (submitted for publication). Another study that used Fidocure identified five genes associated with either a positive or negative prognosis.[40]

*Therapy*: Both SearchLight DNA and Fidocure can also be used for therapeutic guidance. These assays identify mutations that are associated with response to targeted therapies based on published studies that range from preclinical in vitro studies to well-powered in vivo studies validating mutations as proven therapeutic biomarkers. Genomically guided therapies can be used in addition to, in combination with, or in lieu of conventional therapies, depending on the aggressiveness of the patient's cancer, owner's wishes, and/or clinician's professional guidance.

*Screening*: OncoK9 is a multi-cancer early detection test for the detection and characterization of cancer-associated genomic alterations. It is intended for use in dogs that are at higher risk of cancer.

### *Application*

#### *HOW does one apply these tests in practice?*

*Case selection*: Any cases that are at risk of developing cancer (such as older dogs and/or predisposed breeds) could benefit from early screening before the development of clinical signs. For diagnostic elucidation, cases that remain equivocal after initial pathologic (cytologic or histologic) evaluation could benefit from PCR tests that specifically evaluate cancers on the list of differential diagnoses and/or from SearchLight DNA. Uncommon cases or cases that do not have definitive diagnoses could also benefit from SearchLight DNA or Fidocure, which can provide prognostic information based on the identification of mutations in specific prognostic genes. Dogs that need more aggressive therapy or have failed or cannot receive conventional therapy should also consider SearchLight DNA and Fidocure for selection of targeted therapeutic options.

*Sample types and unique collection methods*: PARR can be performed from either FFPE tissues or aspirates (depending on the providing company or institution). PCR for c-MYC mutation (to evaluate for TVT) and C-kit mutation (to evaluate for MCT/GIST/melanoma) can be performed on FFPE/formalin-fixed/fresh tissues or aspirates. PCR for PTPN11 mutation (to evaluate for histiocytic sarcoma) requires whole blood in ethylenediaminetetraacetic acid (EDTA) tubes. PCR for BRAF mutation (to evaluate for urothelial carcinomas) requires a free-caught urine sample into a dedicated CADET *BRAF* urine specimen container that contains a stabilizing agent. OncoK9 requires a peripheral whole blood sample that is collected into specialized blood collection tubes designed to prevent white blood cell lysis and cfDNA degradation. Fidocure is performed on FFPE samples. SearchLight DNA can be performed on FFPE tissue, FNAs, and most sample types in which sufficient neoplastic cellularity can be confirmed by internal pathology review, such as spun-down urine and effusions, on a case-by-case basis.

#### *WHO can use these tests?*

All veterinarians, including but not limited to those involved with primary care, emergency care, shelter medicine, and specialty care, have equal and easy access to all genomic assays.

#### *For WHAT cancers should these tests be performed?*

Because the purpose of the PCR-based tests is to facilitate differentiation of cancer types that may be morphologically difficult to distinguish, these tests should be considered after the differential diagnoses have been narrowed to include the cancer types for which the PCR assay is proposed to facilitate in diagnosing. SearchLight DNA and Fidocure are designed to include all cancer types and can therefore be performed on all cancers for which the sample type is accepted.

### *Therapeutic Options Guided by Genomic Analysis*

Multiple targeted therapeutics, for which there are pharmacokinetic and safety data, are currently available to veterinarians from at least one major compounding pharmacy in the United States (**Table 1**).

### *Limitations*

There are potential limitations to these assays. By only evaluating one or two genes in the PCR-based assays, we may be missing other critical genes that could have

**Table 1**
**Targeted therapies currently available for dogs**

| Targeted Therapy | Suggested NOAEL[a] | Typical Starting Dose[b] | Possible Clinical Signs[c] | Notable Laboratory Abnormalities[d] | Availability to Veterinarians[e] | Availability of Pharmacokinetic Data |
|---|---|---|---|---|---|---|
| Crizotinib | <5 mg/kg/day | 1–2 mg/kg/day | Emesis; watery/mucoid feces | CBC (decreased RBC parameters; increased WBC parameters; increased platelets). Serum biochemistry (increased ALT, AST, ALP, GGT; decreased albumin and calcium) | Yes | Yes |
| Dasatinib | <0.75 mg/kg/day | 0.5 mg/kg/day | Emesis and bloody vomitus; liquid, mucous, and blood in feces | Serum biochemistry (decreased total protein, albumin, globulins; increased ALT) | Yes | Yes |
| Ibrutinib | 1.5 mg/kg/day | 2.5–5 mg/kg/day | Soft feces/diarrhea; emesis; decreased food consumption; reddened or pale gums; raised reddened or white areas on gums; tremors, intermittent convulsion, rigid muscle tone | CBC (increased WBC parameters; increased platelets). Serum biochemistry (increased AST, triglycerides) | Yes | Yes |
| Imatinib | 3 mg/kg/day | 10 mg/kg/day | Emesis | CBC (decreased RBC and WBC parameters). Serum biochemistry (increased ALT) | Yes | Yes |
| Lapatinib | 10 mg/kg/day | 20–30 mg/kg/day | Decreased activity; dehydration; salivation; loose feces; ulcerations in paw and mouth; scabs; emesis | CBC (increased WBC parameters). Serum biochemistry (increased bilirubin, total bile acids, ALP, ALT). Urinalysis (increased bilirubin) | Yes | Yes |

| | | | | | | |
|---|---|---|---|---|---|---|
| Olaparib | <3 mg/kg/day | 2.5–3 mg/kg/day | Lethargy | CBC (decreased RBC and WBC parameters; decreased platelets) | Yes | Yes |
| Palbociclib | <2 mg/kg/day | 0.6 mg/kg/day | Soft feces; red/swollen pinnae | CBC (decreased RBC and WBC parameters, particularly neutrophils; decreased platelets) | Yes | Yes |
| Sirolimus | <0.1 mg/kg/day | 0.1 mg/kg/day | Emesis; diarrhea; anorexia; weight loss; red lesions on gums | CBC (increased WBC parameters) | Yes | Yes |
| Sorafenib | <3 mg/kg/day | 5 mg/kg q12 h | Liquid feces ± blood or mucus; weight loss; sparse hair coat, pustules, alopecia with reddened or bluish skin, dark axillary skin | CBC (decreased RBC parameters; increased WBC parameters; increased platelets). Serum biochemistry (increased ALT, AST, ALP, GGT) | Yes | Yes |
| Toceranib | Not observed (clinical changes noted at all evaluated dose levels) | 2.75 mg/kg every other day | Diarrhea, blood in stool, hemorrhagic diarrhea; anorexia; lethargy; vomiting; nausea; lameness; weight loss; dermatitis; pruritus; tachypnea; localized pain; flatulence; conjunctivitis | CBC (decreased hematocrit; decreased platelets; decreased neutrophils). Serum biochemistry (increased ALT, creatinine, bilirubin; decreased albumin). Urinalysis (urinary tract infection) | Yes | Yes |
| Trametinib | <0.4 mg/m$^2$/day | 0.5 mg/m$^2$/day | Skin lesions, scabs, discharge from and swelling of prepuce or vulva; salivation; gastrointestinal toxicity; lethargy | CBC (anemia, increased reticulocyte count). Serum biochemistry (increased liver enzymes). Urinalysis and/or UPC (increased protein). Blood pressure (increased) | Yes | Yes |

(*continued on next page*)

**Table 1**
**(*continued*)**

| Targeted Therapy | Suggested NOAEL[a] | Typical Starting Dose[b] | Possible Clinical Signs[c] | Notable Laboratory Abnormalities[d] | Availability to Veterinarians[e] | Availability of Pharmacokinetic Data |
|---|---|---|---|---|---|---|
| Vorinostat | 60 mg/kg/day | 22 mg/kg every other day to 30 mg/kg/day | Non-formed or liquid feces; weight loss; dehydration; hypoactive behavior; pale gums; emesis; nausea | CBC (increased or decreased RBC parameters; increased WBC parameters; increased platelets). Serum biochemistry (increased APTT, protein, albumin, creatinine, BUN, BG; decreased P, Na, K, Cl). Urinalysis (increased urine volume, decreased USG, positive occult blood in urine) | Yes | Yes |

*Abbreviations*: ALT, alanine transaminase; ALP, alkaline phosphatase; APTT, activated partial thromboplastin clotting time; AST, aspartate aminotransferase; BG, blood glucose; BUN, blood urea nitrogen; CBC, complete blood count; GGT, gamma-glutamyl transferase; RBC, red blood cell; NDA, new drug application; UPC, urine protein-to-creatinine ratio; USG, urine specific gravity; WBC, white blood cell.

[a] No observed adverse effect level, based on a combination of primary canine publications and canine-specific data in the NDA.

[b] Based on personal communication with multiple veterinary oncologists.

[c] Typically seen at doses significantly higher than NOAEL based on studies performed in the NDA, and these signs could also represent feedback from clinicians using this drug on their patients.

[d] Changes typically seen at doses higher than NOAEL.

[e] Available in at least one major compounding pharmacy in the United States.

*Data from* Refs.[91–104]

therapeutic, diagnostic, or prognostic biomarker associations. For pan-cancer, multigene panels, intra-tumoral heterogeneity may preclude representative sampling for genomic analysis. The efficacy of targeted therapeutics for dogs, whether used alone or in combination with conventional therapies, has yet to be fully explored, although we have early compelling evidence supporting its utility. Finally, for liquid biopsy methods that rely on ctDNA, there is a possibility for insufficient ctDNA in circulation for confident detection and characterization of cancer.

### *Looking Toward the Future*

Emerging evidence of human precision medicine success paves a path toward its broad applications in veterinary medicine. There are already promising early indications for the utility of genomics in cancer monitoring via a noninvasive liquid biopsy method. Genomics can be used to predict future cancer development or to predict disease risk, such as the use of germline BRCA mutations to predict breast cancer risk in women. Genomics can also synergize with and mutually bolster other disciplines, such as immunotherapy, pharmacology, and other "'omics", providing a more comprehensive approach to cancer care. Finally, because sequencing technology continues to advance and become more efficient, we can expect the cost of performing high-throughput genomics to decline with time, allowing more pets to enjoy the many life-saving benefits of precision medicine.

## SUMMARY

1. Precision medicine focuses on the clinical management of the patient based on the individual, not based on population-based findings.
2. There are many successes of precision medicine implementation in human oncology and it is therefore integrated into human cancer management. It is increasingly integrated into canine cancer management, with early evidence of its success.
3. In canine oncology, precision medicine can be integrated into practice as a complement to the conventional approaches to disease characterization, treatment, and monitoring.
4. As genomic profiling costs decrease with time, test costs will decrease, allowing for increased utilization and subsequent improvement of knowledge base from which to make better-informed decisions.
5. Integration of precision medicine in canine oncology has already begun and will only expand in utility and use by veterinarians for improved cancer characterization, enhanced therapy selection, and overall more successful management of canine cancer. As such, practitioners are called to interpret and leverage precision medicine reports for their patients.

## CLINICS CARE POINTS

- Genomics-informed targeted therapies have proven repeatedly successful for human genomic targets, with initial evidence of efficacy in homologous canine targets.
- Several genomic assays, spanning cancer screening to treatment selection, are currently commercially available at the disposal of every veterinarian.
- A growing body of information is available surrounding the safety and early efficacy of targeted therapeutics in dogs.

## DISCLOSURE

W. Hendricks is a full-time employee of Vidium Animal Health; E. Chon and D. Haworth were full time employees of Vidium Animal Health; SearchLight DNA is a product developed and provided by Vidium Animal Health. L. Rodrigues, and G. Post are full-time employees of One Health Company; M. White was a full time employee of One Health Company; FidoCure is a product developed and provided by One Health Company.

## REFERENCES

1. American cancer society. American Cancer Society | Cancer Facts & Statistics Available at: http://cancerstatisticscenter.cancer.org. Accessed August 7, 2023.
2. Comprehensive cancer information. National Cancer Institute. 1980 Available at: http://www.cancer.gov. Accessed August 7, 2023.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646–74.
4. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov 2022;12(1):31–46.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61(5):759–67.
6. About genomics. Genome.gov Available at: http://www.genome.gov/about-genomics. Accessed August 7, 2023.
7. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013;339(6127):1546–58.
8. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer 2005;5(3):172–83.
9. Rawluk J, Waller CF. Gefitinib. Recent Results Cancer Res 2018;211:235–46.
10. Flaherty KT, Yasothan U, Kirkpatrick P. Vemurafenib. Nat Rev Drug Discov 2011;10(11):811–2.
11. Min HY, Lee HY. Molecular targeted therapy for anticancer treatment. Exp Mol Med 2022;54(10):1670–94.
12. Mateo J, Steuten L, Aftimos P, et al. Delivering precision oncology to patients with cancer. Nat Med 2022;28(4):658–65.
13. Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45(10):1113–20.
14. International Cancer Genome Consortium, Hudson TJ, Anderson W, et al. International network of cancer genome projects. Nature 2010;464(7291):993–8.
15. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45(D1):D777–83.
16. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017;2017. https://doi.org/10.1200/PO.17.00011.
17. Tamborero D, Rubio-Perez C, Deu-Pons J, et al. Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations. Genome Med 2018;10(1):25.
18. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 2017;19(1):4–23.

19. Home. Association for Molecular Pathology Available at: http://www.amp.org. Accessed August 7, 2023.
20. Kim ES, Herbst RS, Wistuba II, et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov 2011;1(1):44–53.
21. Tsimberidou AM, Hong DS, Ye Y, et al. Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT): An MD Anderson Precision Medicine Study. JCO Precis Oncol 2017;2017. https://doi.org/10.1200/PO.17.00002.
22. Belin L, Kamal M, Mauborgne C, et al. Randomized phase II trial comparing molecularly targeted therapy based on tumor molecular profiling versus conventional therapy in patients with refractory cancer: cross-over analysis from the SHIVA trial. Ann Oncol 2017;28(3):590–6.
23. Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. Nat Med 2019; 25(5):744–50.
24. Bertucci F, Gonçalves A, Guille A, et al. Prospective high-throughput genome profiling of advanced cancers: results of the PERMED-01 clinical trial. Genome Med 2021;13(1):87.
25. Haslem DS, Van Norman SB, Fulde G, et al. A Retrospective Analysis of Precision Medicine Outcomes in Patients With Advanced Cancer Reveals Improved Progression-Free Survival Without Increased Health Care Costs. J Oncol Pract 2017;13(2):e108–19.
26. Haslem DS, Chakravarty I, Fulde G, et al. Precision oncology in advanced cancer patients improves overall survival with lower weekly healthcare costs. Oncotarget 2018;9(15):12316–22.
27. Shiel RE, Brennan SF, O'Rourke LG, et al. Hematologic values in young pretraining healthy Greyhounds. Vet Clin Pathol 2007;36(3):274–7.
28. Johnstone IB. Canine Von Willebrand's Disease: A Common Inherited Bleeding Disorder in Doberman Pinscher Dogs. Can Vet J 1986;27(9):315–8.
29. Paul AJ, Tranquilli WJ, Seward RL, et al. Clinical observations in collies given ivermectin orally. Am J Vet Res 1987;48(4):684–5.
30. Lindblad-Toh K, Wade CM, Mikkelsen TS, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 2005;438(7069): 803–19.
31. Simpson S, Dunning MD, de Brot S, et al. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics. Acta Vet Scand 2017;59(1):71.
32. Bergholtz H, Lien T, Lingaas F, et al. Comparative analysis of the molecular subtype landscape in canine and human mammary gland tumors. J Mammary Gland Biol Neoplasia 2022;27(2):171–83.
33. Hernandez B, Adissu HA, Wei BR, et al. Naturally Occurring Canine Melanoma as a Predictive Comparative Oncology Model for Human Mucosal and Other Triple Wild-Type Melanomas. Int J Mol Sci 2018;19(2). https://doi.org/10.3390/ijms19020394.
34. Wong S, Ehrhart EJ, Stewart S, et al. Genomic landscapes of canine splenic angiosarcoma (hemangiosarcoma) contain extensive heterogeneity within and between patients. PLoS One 2022;17(7):e0264986.
35. Rodrigues L, Watson J, Feng Y, et al. Shared hotspot mutations in oncogenes position dogs as an unparalleled comparative model for precision therapeutics. Sci Rep 2023;13(1):10935.
36. Wang G, Wu M, Maloneyhuss MA, et al. Actionable mutations in canine hemangiosarcoma. PLoS One 2017;12(11):e0188667.

37. Cronise KE, Das S, Hernandez BG, et al. Characterizing the molecular and immune landscape of canine bladder cancer. Vet Comp Oncol 2022;20(1):69–81.
38. Mochizuki H, Shapiro SG, Breen M. Detection of BRAF Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. PLoS One 2015;10(12):e0144170.
39. Decker B, Parker HG, Dhawan D, et al. Homologous mutation to human BRAF V600E is common in naturally occurring canine bladder cancer—Evidence for a relevant model system and urine-based diagnostic test. Mol Cancer Res 2015;13(6):993–1002.
40. Wu K, Rodrigues L, Post G, et al. Analyses of canine cancer mutations and treatment outcomes using real-world clinico-genomics data of 2119 dogs. npj Precis Oncol 2023;7(1):8.
41. Maeda S, Sakai K, Kaji K, et al. Lapatinib as first-line treatment for muscle-invasive urothelial carcinoma in dogs. Sci Rep 2022;12(1):4.
42. Paoloni MC, Mazcko C, Fox E, et al. Rapamycin pharmacokinetic and pharmacodynamic relationships in osteosarcoma: a comparative oncology study in dogs. PLoS One 2010;5(6):e11013.
43. JU Pontius, Mullikin JC, Smith DR, et al. Initial sequence and comparative analysis of the cat genome. Genome Res 2007;17(11):1675–89.
44. Buckley RM, Davis BW, Brashear WA, et al. A new domestic cat genome assembly based on long sequence reads empowers feline genomic medicine and identifies a novel gene for dwarfism. PLoS Genet 2020;16(10):e1008926.
45. Ludwig L, Dobromylskyj M, Wood GA, et al. Feline Oncogenomics: What Do We Know about the Genetics of Cancer in Domestic Cats? Vet Sci China 2022;9(10). https://doi.org/10.3390/vetsci9100547.
46. Sutter NB, Ostrander EA. Dog star rising: the canine genetic system. Nat Rev Genet 2004;5(12):900–10.
47. Boyko AR, Quignon P, Li L, et al. A simple genetic architecture underlies morphological variation in dogs. PLoS Biol 2010;8(8):e1000451.
48. Lloyd KCK, Khanna C, Hendricks W, et al. Precision medicine: an opportunity for a paradigm shift in veterinary medicine. J Am Vet Med Assoc 2016;248(1):45–8.
49. Katogiritis A, Khanna C. Towards the Delivery of Precision Veterinary Cancer Medicine. Vet Clin North Am Small Anim Pract 2019;49(5):809–18.
50. Garden OA, Volk SW, Mason NJ, et al. Companion animals in comparative oncology: One Medicine in action. Vet J 2018;240:6–13.
51. LeBlanc AK, Mazcko CN. Improving human cancer therapy through the evaluation of pet dogs. Nat Rev Cancer 2020;20(12):727–42.
52. Aupperle-Lellbach H, Grassinger J, Hohloch C, et al. Diagnostic value of the BRAF variant V595E in urine samples, smears and biopsies from canine transitional cell carcinoma. Tierarztl Prax Ausg K Kleintiere Heimtiere 2018;46(5):289–95.
53. Naka N, Ohsawa M, Tomita Y, et al. Prognostic factors in angiosarcoma: a multivariate analysis of 55 cases. Journal of surgical. 1996. https://onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1096-9098(199603)61:3%3C170::AID-JSO2%3E3.0.CO;2-8.
54. Wang G, Wu M, Durham AC, et al. Molecular subtypes in canine hemangiosarcoma reveal similarities with human angiosarcoma. PLoS One 2020;15(3):e0229728.
55. Wang G, Wu M, Durham AC, et al. Canine Oncopanel: A capture-based, NGS platform for evaluating the mutational landscape and detecting putative driver mutations in canine cancers. Vet Comp Oncol 2022;20(1):91–101.

56. Jahr S, Hentze H, Englisch S, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61(4):1659–65.
57. Schaefer DMW, Forman MA, Kisseberth WC, et al. Quantification of plasma DNA as a prognostic indicator in canine lymphoid neoplasia. Vet Comp Oncol 2007; 5(3):145–55.
58. Tagawa M, Shimbo G, Inokuma H, et al. Quantification of plasma cell-free DNA levels in dogs with various tumors. J Vet Diagn Invest 2019;31(6):836–43.
59. Kim J, Bae H, Ahn S, et al. Cell-Free DNA as a Diagnostic and Prognostic Biomarker in Dogs With Tumors. Front Vet Sci 2021;8:735682.
60. Prouteau A, Denis JA, De Fornel P, et al. Circulating tumor DNA is detectable in canine histiocytic sarcoma, oral malignant melanoma, and multicentric lymphoma. Sci Rep 2021;11(1):877.
61. Flory A, Kruglyak KM, Tynan JA, et al. Clinical validation of a next-generation sequencing-based multi-cancer early detection "liquid biopsy" blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study. PLoS One 2022;17(4):e0266623.
62. Tagawa M, Aoki M. Clinical utility of liquid biopsy in canine oral malignant melanoma using cell-free DNA. Front Vet Sci 2023;10. https://doi.org/10.3389/fvets.2023.1182093.
63. Wilson-Robles HM, Bygott T, Kelly TK, et al. Evaluation of plasma nucleosome concentrations in dogs with a variety of common cancers and in healthy dogs. BMC Vet Res 2022;18(1):329.
64. Caputo V, Ciardiello F, Corte CMD, et al. Diagnostic value of liquid biopsy in the era of precision medicine: 10 years of clinical evidence in cancer. Explor Target Antitumor Ther 2023;4(1):102–38.
65. Castro-Giner F, Gkountela S, Donato C, et al. Cancer Diagnosis Using a Liquid Biopsy: Challenges and Expectations. Diagnostics 2018;8(2). https://doi.org/10.3390/diagnostics8020031.
66. Mathur S, Sutton J. Personalized medicine could transform healthcare. Biomed Rep 2017;7(1):3–5.
67. Manzari MT, Shamay Y, Kiguchi H, et al. Targeted drug delivery strategies for precision medicines. Nat Rev Mater 2021;6(4):351–70.
68. Wanebo HJ, Argiris A, Bergsland E, et al. Targeting growth factors and angiogenesis; using small molecules in malignancy. Cancer Metastasis Rev 2006; 25(2):279–92.
69. Normanno N, Gullick WJ. Epidermal growth factor receptor tyrosine kinase inhibitors and bone metastases: different mechanisms of action for a novel therapeutic application? Endocr Relat Cancer 2006;13(1):3–6.
70. London CA. Tyrosine kinase inhibitors in veterinary medicine. Top Companion Anim Med 2009;24(3):106–12.
71. Hahn KA, Ogilvie G, Rusk T, et al. Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med 2008;22(6):1301–9.
72. Joo WD, Visintin I, Mor G. Targeted cancer therapy–are the days of systemic chemotherapy numbered? Maturitas 2013;76(4):308–14.
73. Imai K, Takaoka A. Comparing antibody and small-molecule therapies for cancer. Nat Rev Cancer 2006;6(9):714–27.
74. Rue SM, Eckelman BP, Efe JA, et al. Identification of a candidate therapeutic antibody for treatment of canine B-cell lymphoma. Vet Immunol Immunopathol 2015;164(3–4):148–59.

75. Maekawa N, Konnai S, Ikebuchi R, et al. Expression of PD-L1 on canine tumor cells and enhancement of IFN-γ production from tumor-infiltrating cells by PD-L1 blockade. PLoS One 2014;9(6):e98415.
76. Kumar SR, Kim DY, Henry CJ, et al. Programmed death ligand 1 is expressed in canine B cell lymphoma and downregulated by MEK inhibitors. Vet Comp Oncol 2017;15(4):1527–36.
77. Coy J, Caldwell A, Chow L, et al. PD-1 expression by canine T cells and functional effects of PD-1 blockade. Vet Comp Oncol 2017;15(4):1487–502.
78. Choi JW, Withers SS, Chang H, et al. Development of canine PD-1/PD-L1 specific monoclonal antibodies and amplification of canine T cell function. PLoS One 2020;15(7):e0235518.
79. Igase M, Nemoto Y, Itamoto K, et al. A pilot clinical study of the therapeutic antibody against canine PD-1 for advanced spontaneous cancers in dogs. Sci Rep 2020;10(1):18311.
80. Maekawa N, Konnai S, Nishimura M, et al. PD-L1 immunohistochemistry for canine cancers and clinical benefit of anti-PD-L1 antibody in dogs with pulmonary metastatic oral malignant melanoma. npj Precis Oncol 2021;5(1):10.
81. Minoli L, Licenziato L, Kocikowski M, et al. Development of Monoclonal Antibodies Targeting Canine PD-L1 and PD-1 and Their Clinical Relevance in Canine Apocrine Gland Anal Sac Adenocarcinoma. Cancers 2022;14(24). https://doi.org/10.3390/cancers14246188.
82. Whilde J, Martindale MQ, Duffy DJ. Precision wildlife medicine: applications of the human-centred precision medicine revolution to species conservation. Glob Chang Biol 2017;23(5):1792–805.
83. Sakthikumar S, Facista S, Whitley D, et al. Standing in the canine precision medicine knowledge gap: Improving annotation of canine cancer genomic biomarkers through systematic comparative analysis of human cancer mutations in COSMIC. Vet Comp Oncol 2023. https://doi.org/10.1111/vco.12911.
84. Chon E, Wang G, Whitley D, et al. Genomic tumor analysis provides clinical guidance for the management of diagnostically challenging cancers in dogs. J Am Vet Med Assoc 2023;261(5):668–77.
85. Chon E, Sakthikumar S, Tang M, et al. Novel genomic prognostic biomarkers for dogs with cancer. J Vet Intern Med 2023;37(6):2410–21.
86. O'Kell AL, Lytle KM, Cohen TA, et al. Clinical experience with next-generation sequencing-based liquid biopsy testing for cancer detection in dogs: a review of 1,500 consecutive clinical cases. J Am Vet Med Assoc 2023;261(6):827–36.
87. Rafalko JM, Kruglyak KM, McCleary-Wheeler AL, et al. Age at cancer diagnosis by breed, weight, sex, and cancer type in a cohort of more than 3,000 dogs: Determining the optimal age to initiate cancer screening in canine patients. PLoS One 2023;18(2):e0280795.
88. Ramón Y, Cajal S, Sesé M, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. J Mol Med 2020;98(2):161–77.
89. Sung JY, Shin HT, Sohn KA, et al. Assessment of intratumoral heterogeneity with mutations and gene expression profiles. PLoS One 2019;14(7):e0219682.
90. Sutherland KD, Visvader JE. Cellular Mechanisms Underlying Intertumoral Heterogeneity. Trends Cancer Res 2015;1(1):15–23.
91. Xalkori® (Crizotinib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 202570, 2011.
92. Sprycel® (Dasatinib); European Medicines Agency, European public assessment report (EPAR) Scientific Discussion, 2006.

93. Davis, Hofmann NE, Li G, et al. A case study of personalized therapy for osteosarcoma. Pediatr Blood Cancer 2013;60(8):1313–9.
94. Imbruvica® (Ibrutinib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 205552 (capsule), February 2014.
95. Honigberg, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. Proc Natl Acad Sci U S A 2010;107(29):13075–80.
96. Gleevec® (Imatinib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 021335 (capsule),2001.
97. Tykerb® (Lapatinib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 022059, 2007.
98. Lynparza® (Olaparib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 206162 (capsule), 2014.
99. Ibrance® (Palbociclib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 207103 (capsule), 2015.
100. Rapamune® (Sirolimus/Rapamycin); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 021083, 1999.
101. Nexavar® (Sorafenib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 021923, 2005.
102. Mekinist® (Trametinib); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 204114, 2013.
103. Takada MMS, Jones A, Onsager A, et al. Phase I clinical trial to evaluate the tolerability of trametinib in dogs with cancer, In: Veterinary Cancer Society Annual Meeting, 2022; Norfolk, VA, 44.
104. Zolinza® (Vorinostat); Pharmacology Review. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, NDA 021991, 2006.