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REVIEW

Molecular pathology in the cancer clinic – where are we now and where are we headed?

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Molecular pathology is a developing sub-microscopic discipline of pathology that studies the effects of molecular variations and mutations on disease processes. The ultimate goal of molecular pathology in cancer is to predict risk, facilitate diagnosis and improve prognostication based on a complete understanding of the biological impact of specific molecular variations, mutations and dysregulations. This knowledge will provide the basis for customised cancer treatment, so-called precision medicine. Rapid developments in genomics have placed this field at the forefront of clinical molecular pathology and there are already a number of well-established genetic tests available for clinical use including PCR of antigen receptor rearrangement and KIT mutational analysis. Moving beyond tests assessing a single gene, there are significant research efforts utilising genomics to predict cancer risk, forecast aggressive behaviour and identify druggable mutations and therapeutic biomarkers. Researchers are also investigating the use of circulating cells and nucleic acid for clinically useful low morbidity genomic assessments. If we are to realise the full potential of molecular pathology and precision medicine there are a number of challenges to overcome. These include developing our understanding of the underlying biology (in particular intra-tumoural heterogeneity), methodological standardisation of assays, provision of adequate infrastructure and production of novel therapeutics backed by high-quality clinical data supporting the precision medicine approach. The era of molecular pathology holds the potential to revolutionise veterinary cancer care, but its impact on clinical practice will depend upon the extent to which the inherent challenges can be overcome.

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INTRODUCTION

Molecular pathology is a developing sub-microscopic discipline of pathology. The fundamental principle of molecular pathology is that studying the effects of molecular variations on cellular physiology and clinical phenotype will yield insights useful to clinicians managing cancer patients. Key goals of studying molecular pathology are facilitating diagnosis and prognosis by allowing more certain identification of disease subtypes and ultimately building our understanding of the significance of molecular variations as the basis for tailoring of clinical treatments to individuals – so-called precision medicine (see Fig 1).

Molecular pathology has at its core a cross-disciplinary philosophy that utilises techniques from fields including genomics, epigenomics, transcriptomics, proteomics and metabolomics. In this broad sense, the field started with the development of immunohistochemistry (IHC) by Marrack and subsequently Coons in the 1930s and 1940s (Childs 2014). Over recent years the rapid development and increased accessibility of techniques such as PCR, microarrays

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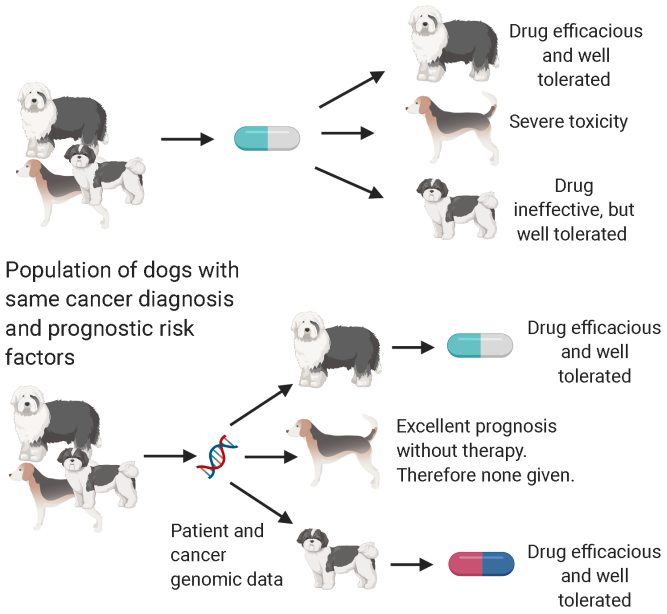

FIG 1. An idealised example of how precision medicine might improve management of cancer patients. Created with BioRender.com

and more recently, next-generation sequencing in combination with a greater genetic knowledge base after the publication of an annotated version of the canine genome sequence in 2005 (Lindblad-Toh *et al.* 2005) has led to a focus on genomics and transcriptomics.

There is now a large and rapidly expanding volume of work in this field with a number of excellent reviews such as that by Morris (2016). Our goal in this review is not to re-capitulate every discovery in the field, but rather to outline what is available in the clinic today, explore the clinical opportunities offered by ongoing research efforts and to examine the broader issues and challenges to widespread adoption of molecular pathology and precision medicine by veterinary surgeons treating cancer.

WHAT IS THE CASE FOR DEVELOPING MOLECULAR PATHOLOGY TO SUPPORT THE ONCOLOGY CLINIC?

Over the last 20 years, significant progress has been made in identifying breeds with particular cancer predispositions, refining conventional diagnostic techniques based on phenotype using cytology, histology and IHC and improving prognostication via increasing availability of advanced imaging techniques and publication of a plethora of reports categorising patient and disease characteristics against patient outcome. Nonetheless, there is still huge variation in outcomes of seemingly similar patients and it is unlikely that veterinary oncology can progress much further towards meeting improving patient care with the current toolkit. There are perhaps four broad challenges that could be met through the use of molecular pathology (summarised in Fig 2).

Cancer risk prediction and early diagnosis of cancer

Cancer is a disease with both genetic and environmental risk factors. Identification of the genetic predispositions would allow owners to understand risk factors before procuring a pet and let them consider in advance how they would manage their pet should they unfortunately develop cancer. Steps could also be taken to breed high-risk genotypes out of a particular breed line.

Beyond risk prediction, for most cancers early diagnosis is associated with a better outcome. However, clinical signs of cancer only often develop once the disease is advanced. Consequently, the development of accurate screening tools suitable for low morbidity assessment of at risk pets should lead to improved outcomes.

Improved prediction of which patients will benefit from adjuvant therapy to prevent tumour recurrence or metastatic disease

Currently, clinicians have to make treatment and follow-up decisions based on data with significant uncertainty. Improved tools for assessment of risk would allow some patients to avoid unnecessary adjuvant therapy. Such tests would also facilitate research by allowing more accurate identification of the disease sub-types allowing researchers to focus their efforts on the most problematic variants.

Reducing the morbidity and cost of diagnosis and monitoring of cancer

The advances over the last 30 years have made gold standard clinical oncology cost prohibitive for many clients; one advantage of molecular pathology techniques is that their sensitivity may allow clinicians to make a diagnosis at a lower cost or with the use of fewer invasive tests.

Matching cancer patients with therapeutics which are well tolerated and efficacious against their particular tumour sub-type

Improved understanding of the underlying biology of pet cancers should allow identification of biomarkers for more efficacious and less toxic use of conventional therapies, more successful use of current therapies such as tyrosine kinase inhibitors, and development of novel therapies targeting particular mutations.

WHAT IS AVAILABLE IN THE CLINIC NOW?

The currently available molecular tests are focussed on either easing diagnosis or pharmacogenomically guided treatment selection. They include tests to reduce uncertainty in cases of possible lymphoma, reduce cost and morbidity in the diagnosis of transitional cell carcinoma of the bladder, and guide treatment choice in lymphoma and mast cell tumour (MCT). A summary of the available tests and some relevant characteristics is shown in Table 1.

Diagnosis of lymphoma using PARR

PCR for antigen receptor rearrangement (PARR) is probably the most commonly used molecular diagnostic test in veterinary oncology today. A common clinical problem is the differentiation of reactive and neoplastic lymphocyte populations; espe-

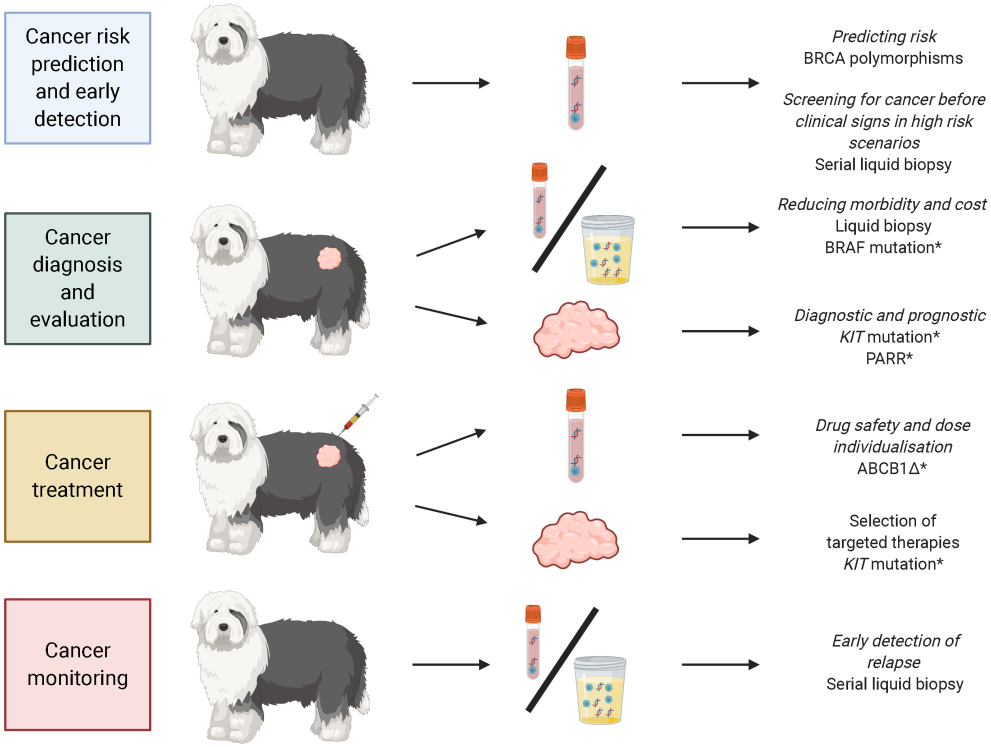

FIG 2. Molecular pathology has the potential to assist the clinician at each stage of cancer management. *Assays are already commercially available. Created with BioRender.com

cially in settings in which collecting tissue biopsies is challenging due to cost or morbidity. Clinical uses include aiding in the differentiation of follicular lymphomas and inflammation, differentiating chronic enteropathies from lymphoma and separating thymoma from mediastinal lymphoma (Burnett *et al.* 2003, Ohmura *et al.* 2017, Vessieres *et al.* 2018). A significant strength of PARR is that it can often be performed on previously collected FNA and biopsy samples which have yielded an equivocal result thus obviating the need for further sampling procedures. PARR also allows for basic immunophenotyping of lymphoma into B or T cell sub-types (Burnett *et al.* 2003). Confusion can arise when PARR is positive for both B and T cell rearrangements and with certain intracellular infections (Burnett *et al.* 2003, Valli *et al.* 2006, Weiss *et al.* 2011). Moreover, flow cytometry is superior to PARR for determining lymphoma phenotype (compared to the gold standard of IHC) (Thalheim *et al.* 2013), but is not as readily available and requires rapid delivery of the samples to the lab.

Cytological, formalin-fixed paraffin-embedded and fresh tissue preparations can be used for PARR (Burnett *et al.* 2003, Schopper *et al.* 2017, Vessieres *et al.* 2018). DNA extracted from a sample containing lymphocytes is used as a template for PCR amplification of part of the T cell or B cell receptor. The product DNA is then assessed to determine if it is of a single length and hence the DNA is derived from a population of a single lymphocyte clone (*i.e.* they all have the same receptor) and are therefore neoplastic or alternatively of multiple lengths and hence representing a mixed lymphocyte population consistent with inflammation. Rarely neoplastic lymphocytes can be bi-clonal (*i.e.* two products are amplified by PCR).

The area of DNA targeted is the complementarity determining region 3 (CDR3) of the T cell receptor genes or the immunoglobulin heavy chain genes (B cell) (Burnett *et al.* 2003). Nascent lymphocytes carry a hypervariable genetic region which could code for multiple variants of the receptor component genes (VDJ genes) (Burnett *et al.* 2003). During recombination and selection the hypervariable section is shed such that each selected lymphocyte carries a single VDJ (IgH) or VJ (TCRγ) configuration (Burnett *et al.* 2003) and thus receptor shape (see Fig 3 for a conceptualisation of PARR). The necessary extreme diversity of antigen recognition is ensured by the number of receptor variants across the population of lymphocytes. For each assay several sets of PCR primers are used such that they will hybridise to conserved parts of the V and J regions flanking the hypervariable area, consequently DNA from each lymphocyte will produce a (near) unique PCR product (Burnett *et al.* 2003, Morris 2016). Therefore, a single PCR product indicates the presence of a clonally expanded population of lymphocytes. In most situations, this indicates the presence of lymphoma (or other lymphoproliferative disease), but infectious disease (especially Ehrlichiosis) can lead to false positive results (Vernau & Moore 1999, Burnett *et al.* 2003). Various methodologies such as Sanger sequencing (with or without cloning), capillary gel electrophoresis and high-resolution melting curve analysis have been developed in an effort to enhance the precision of the data produced by PARR beyond that yielded by gel electrophoretic size separation alone (Burnett *et al.* 2003, Goto-Koshino *et al.* 2015, Waugh *et al.* 2016, Schopper *et al.* 2017).

As highlighted above, several laboratories have developed a variety of PARR methodologies and as yet there is no broadly

Table 1. Molecular pathology tests currently available for use in the cancer clinic

Test	Cancer	Use	Specimen type	Method	Detection	Advantages	Disadvantages
PARR	Lymphoid neoplasia	Diagnosis and monitoring	Cytological smear Tumour tissue Fluid specimens	PCR	Clonality: IgH or TCRγ gene rearrangements	Moderate to high sensitivity (dog: 75 to 90%; cat: 65 to 89%) and specificity (dog: 92%; cat: 96%)	1. Cost 2. Long turnaround time† 3. Results often required clinical interpretation 4. Lack of standardised laboratory practices
BRAF mutation detection assay	Urothelial and prostatic carcinoma	Diagnosis and monitoring	Urine Tumour tissue Cytological smears	ddPCR	Thymine-to-adenine transversion in exon 15 (BRAF V595E variant)	1. Cost 2. Non-invasive 3. Short turnaround time‡ 4. Moderate to high sensitivity (80 to 95%) and specificity (85 to 100%)	1. Availability limited to a few providers
BCR-ABL translocation detection assay	Chronic myeloid leukaemia	Diagnosis, treatment and monitoring	Heparin and EDTA whole blood Cytological smear	FISH RT-PCR	Translocation between CFA 9 (ABL) and CFA 26 (BCR)	1. Short turnaround time‡ 2. Mutation supports the use of imatinib	1. Cost 2. Limited information regarding availability at time of writing
ABCB1 polymorphism analysis	Any	Treatment	Whole blood EDTA Buccal swabs	PCR	4-base pair deletion (ABCB1-1Δ)	1. Cost 2. Predicts chemotherapy tolerability	1. Long turnaround time† 2. Variable sensitivity across laboratories
c-kit mutation analysis	Cutaneous mast cell tumour	Treatment and prognosis	Tumour tissue Cytological smear	PCR	ITD in exon 8 and 11 of the c-kit gene most commonly Point mutations in exon 9 or 17	1. Exon 11 ITD relatively common mutation (30 to 50%) 2. May support use of TKI 3. Provides prognostic information	1. Cost 2. Long turnaround time† 3. Significant proportion of MCTs with exon 11 ITD do not respond to TKI 4. Exon 17 mutations unlikely to respond to TKIs based on human experience 5. Relationship between mutation and oncogenic/prognostic effects poorly explored, especially beyond exon 11 ITDs
LINE-c-myc assay	Transmissible venereal tumour	Diagnosis and monitoring	Tumour tissue Cytological smear	PCR	LINE-c-myc rearrangement	1. High sensitivity (100%) and moderate specificity (82%) 2. Small amount of DNA needed	3. Not widely available

PARR Polymerase chain reaction for antigen receptor rearrangement, ABCB1 ATP binding cassette subfamily B member 1, PCR Polymerase chain reaction, ddPCR Digital droplet polymerase chain reaction, CFA Dog chromosome, ITD Intratandem duplication, TCRγ T-cell receptor gamma, IgH Immunoglobulin heavy chain
†Often more than 7 days or more for most laboratories
‡Usually less than 7 days

agreed standardised methodology for PARR. Recent studies of PARR in canine lymphoma have reported sensitivity of greater than 80% (Goto-Koshino *et al*. 2015, Waugh *et al*. 2016, Vessieres *et al*. 2018) and up to 98% (Gentilini *et al*. 2009). In one study, the sensitivity was lower in intestinal compared to lymph node assessments (Goto-Koshino *et al*. 2015). The specificity was 98.7% in another study (Waugh *et al*. 2016), although this was undertaken in the UK where Ehrlichiosis is rarely diagnosed. These data highlight that clinicians must consider how the presentation of the case in question and their laboratory's methodology compares to the published literature when interpreting results.

So far PARR in cats has not been as successful as in dogs with a sensitivity of >79% reported for T cell lymphoma in most studies, but a range of 34 to 89% reported for B cell lymphoma. A recent paper evaluated whether adding assessment of incomplete recombination (IgH DJ) and additional regions of the B cell receptor (Ig Kappa, Kappa deleting element and Ig lambda light chain) and found this improved the sensitivity of PARR from 50 to 87% in the group of lymphomas assessed (Rout *et al*. 2019).

Lowering cost of diagnosis of TCC by identification of the BRAF mutation

The recently commercialised BRAF mutation assay supports the diagnosis of urothelial carcinoma/transitional cell carcinoma (UC) from a 40 to 60 mL free catch urine sample. Use of a free catch samples allows clinicians to make a diagnosis of UC, with reduced cost and morbidity compared to traditional biopsy methods such as traumatic catheterisation, in patients with consistent clinical signs (and ideally imaging). The ease of the test also facilitates serial sampling of pets who are considered at risk due to breed or potentially for prognostic purposes.

This test is based on the identification of an exon 15 mutation in the *BRAF* coding sequence. In humans, a wide variety

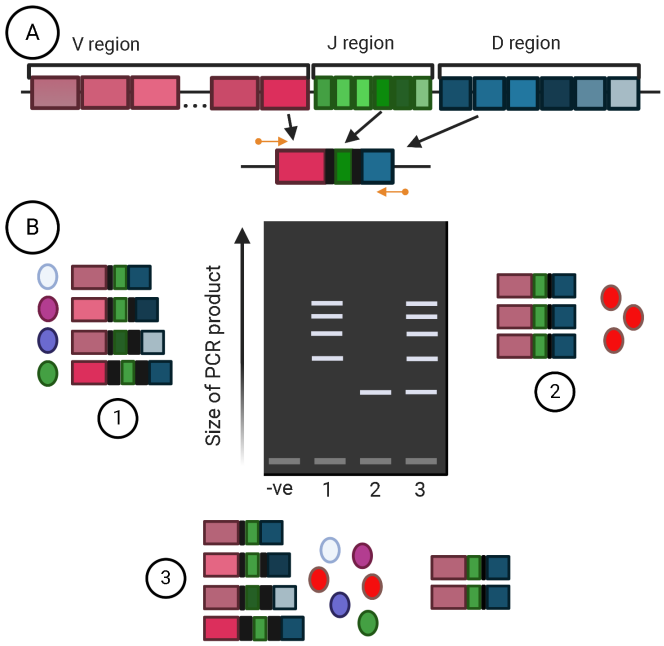

FIG 3. A simplified conceptual outline of the basis for clonality testing and interpretation of results using the B cell IgH as an example. (A) The huge diversity in lymphocyte receptor affinities is acquired through the random recombination during the process of V(D)J recombination. During the joining process a small number of bases is added randomly to between V&D and D&J (shown in black). Consequently lymphocytes have different lengths of VDJ. A selection of flanking primer sets designed to bind in a less variable region can be used to amplify most of the variants by PCR. The length of the variants can then be separeted by length using electrophoresis. (B) A gel electrophoresis shows three hypothetical scenarios. 1. A polyclonal selection of lymphocytes has been assessed, consistent with inflammation. 2. A clonal population of lymphocytes has been assessed, consistent with lymphoma. 3. A small neoplastic population is present, but cannot be identified as it is below the limit of detection of the assay, hence a false-negative result would be produced. Estimates of limit of detection suggest malignant clones present at a ratio of 1:10 000 or more can be detected with the most sensitive methods. Methodology used by individual laboratories will vary. The gel electrophoresis approach here is qualitative with respect clone numbers and has poor resolution between clones of very similar lengths, but semi-quantitative and quantitative methdologies with greater resolution (such as capillary electrophoresis) are increasingly coming into clinical use. Created with BioRender.com.

of tumours including cutaneous melanoma and thyroid cancer have been shown to carry mutations of the *BRAF* gene that lead to constitutive activation of BRAF and consequently the MAPK pathway (Mochizuki *et al.* 2015). The most common mutation (>90%) is in exon 15 and leads to a V595E protein mutation in the gene. Recently a similar mutation was found to be very common in canine urothelial/transitional cell carcinoma of the bladder; occurring in >80% of prostate and UC (Decker *et al.* 2015, Mochizuki *et al.* 2015). Melanoma and cutaneous squamous cell carcinoma infrequently (<10%) carried the mutation and it was not detected in the round cell tumours or sarcomas evaluated (Mochizuki *et al.* 2015).

Soon after the first reports of *BRAF* mutations in UC, it was reported that mutated *BRAF* DNA could be detected in urine from these dogs (Decker *et al.* 2015, Mochizuki *et al.* 2015). Evaluations reported so far suggest the mutation is not present in normal bladder or prostate or inflammatory conditions of the bladder or urethra (Mochizuki *et al.* 2015) and the concordance between *BRAF* mutation results in the tumour and urine is reported to be 100%. Various assays have been reported including PCR followed by Sanger sequencing or restriction fragment length polymorphism assays and digital droplet PCR (Decker *et al.* 2015). The digital droplet PCR method appears more sensitive than Sanger sequencing (Mochizuki *et al.* 2015) and the commercially available CADET® *BRAF* assay uses this method. The reported sensitivity of this method for UC is 85% (Wiley *et al.* 2019). The test is inexpensive compared to a catheter biopsy-based approach and the results are normally available within a week, however as the sensitivity is imperfect an individual case-based interpretation is essential to avoid false-negative findings. The company behind the CADET® *BRAF* assay (Sentinel Biomedical, USA) also market the CADET® *BRAF*-PLUS assay which additionally identifies copy number aberrations in segments of canine chromosomes 13, 19 and 36. These additions are reported to raise the sensitivity to 99.9% for UC (Wiley *et al.* 2019).

Using pharmacogenetics of ABCB1 to guide chemotherapy prescribing

Pharmacogenetics is the study of how genetic polymorphisms can affect both the therapeutic and adverse effect profile of drugs. Much is yet to be learnt about how genetic variations influence responses to therapies in pet species. So far the clinically actionable biomarker in oncology is the ABCB1 mutation, the presence of which can be determined using DNA extracted from a standard blood sample and will direct the clinician to adjust their chemotherapy protocols if the ABCB1-1Δ mutation is present.

The gene *ABCB1* (also known as *MDR-1*) encodes the cell membrane efflux pump P-glycoprotein. P-glycoprotein is expressed at the blood brain barrier, intestinal barrier and hepatic and renal excretory surfaces and is important in the excretion of many biologically active compounds. P-glycoprotein has a wide range of drug substrates including commonly used chemotherapy drugs in the vinca alkaloid and anthracycline classes (specifically vincristine and doxorubicin have been evaluated in dogs) (Mealey 2004). ABCB1-1Δ is a 4 base pair deletion that leads to several premature stop codons in the *ABCB1* gene sequence and consequently a truncated and ineffective protein. The functional outcome is significantly reduced biliary excretion of substrate drugs (Coelho *et al.* 2009) and a defective blood brain barrier in respect to these medicines. Over 50% of Collies and Australian Shepherds carry at least one allele with this polymorphism; significant proportions of other breeds such as German Shepherd Dogs and Shetland Sheepdogs are also affected in some studies. It is, however, worth noting that there is some variation in frequency in different breeds by location therefore clinicians should review the data most relevant to their region (Mealey & Meurs 2008, Gramer *et al.* 2011, Tappin *et al.* 2012, Monobe *et al.* 2015, Firdova *et al.* 2016).

Clinically, dogs carrying the ABCB1-1Δ polymorphism are more likely to develop neutropenia and thrombocytopenia when treated with vincristine (Mealey 2004) and most likely anthracyclines also (given that it is believed some of the excretion pathways are shared by both groups of drugs). Therefore, in

predisposed breeds, ABCB1 genotyping before the administration of substrates will allow the clinician to minimise toxicity and treatment delays (Mealey *et al.* 2003, Mealey *et al.* 2008). For example, during treatment of lymphoma, in these breeds, planned CHOP or COP protocol can be reordered to administer only cyclophosphamide and prednisolone on week 1 to reduce risk and avoid treatment delays whilst awaiting assay results. In affected dogs, alterations to treatment strategies include modifying standard protocols to increase the use of non-substrate drugs (*e.g.* alkylators), changing to alkylator only protocols (such as LPP for lymphoma) or reducing the dosage of vinca alkaloids and anthracyclines. Dose reductions appear effective for vinca alkaloids; however, there is a risk that the magnitude of dose reduction required to avoid adverse effects may mean that only an ineffectual dose could be administered. This has been highlighted in a study modelling doxorubicin administration in $ABCB1_{null}$ patients. The results suggested that these patients are also more likely to suffer significant GI toxicosis than $ABCB1_{wt}$ patients and it may not be possible to identify a safe and efficacious dose for $ABCB1_{null}$ patients (Gustafson & Thamm 2010).

Choosing medical therapy for MCT based on the presence of c-kit mutation

The tyrosine kinase receptor c-kit is activated by its ligand stem cell factor and acts as the primary growth factor for mast cells. It is also expressed in a range of other cell types including melanocytes, mammary cells, Purkinje cells, interstitial cells of Cajal, neuroblastic cells of the retina, Leydig cells and spermatogonia, mature dendritic cells and most (but not all) haematopoietic stem cells (Rico-Vargas *et al.* 1994, Huss *et al.* 1995, Reguera *et al.* 2000, Morini *et al.* 2004, Gilfillan & Rivera 2009, Usher *et al.* 2009, Grieco *et al.* 2010, Avila-Garcia *et al.* 2012, Lennartsson & Ronnstrand 2012).

Mutations in *KIT* have been reported in feline and canine cancers. Some of these mutations lead to constitutive activation of c-kit and are associated with poorer outcomes in MCT patints. Mutations of particular relevance include those in the sequence coding in for the inhibitory juxtamembrane region (JMR, exons 9 and 11) and those in the second kinase domain (exon 17). Initial reports demonstrated that these mutations lead to ligand-independent activation (Furitsu *et al.* 1993) and thus uncontrolled cell growth (Kitayama *et al.* 1995) [one of Hanahan and Weinberg's hallmarks of cancer (Hanahan & Weinberg 2011)].

c-kit mutations appear to play a central role in oncogenesis and progression in a substantial proportion of MCTs. Depending upon report, 13 to 50% (most commonly 25 to 35%) of canine MCTs have internal tandem duplications (duplication and insertion of a copy of small section neighbouring DNA) in the JMR resulting in constitutive activation (Downing *et al.* 2002, Webster *et al.* 2006, Letard *et al.* 2008). Unsurprisingly, together with staging, grading, cell proliferation analyses and margin assessment, c-kit IHC and mutations in exon 11 of *KIT* are an established negative prognostic indicator in canine cutaneous MCTs (Downing *et al.* 2002, Zemke *et al.* 2002, Webster *et al.* 2006, Letard *et al.* 2008). This is particularly relevant in dogs with high-grade MCTs but also in dogs with low and intermediate grade MCTs showing aggressive behaviour, where combining information from histology, proliferation indices (mitotic index and Ki-67) and c-kit assessment may offer increased levels of evidence during prognostication.

The concept of inhibiting a constitutively activated mutated oncogene with a targeted molecule (in this case a tyrosine kinase inhibitor) is an attractive therapeutic option in the context of the "Hallmarks of Cancer." Indeed, small molecular inhibitors have been an important development in human clinical oncology. These drugs act by 'filling' an ATP binding pocket on the target molecule and thus competitively inhibiting downstream signalling from kinase molecules. They are semi-specific affecting only target molecules and those with significant structural similarity; typically the closest in evolutionary terms to the main target molecule. As they are competitive inhibitors, it is logical that they will be most effective in tumours with elevated signalling activity via the target tyrosine kinase. In human oncology, these drugs are often licensed only for use in patients whose tumours have been shown to have a mutation leading to constitutive activation of the target molecule. Consistent with this approach initial reports showed more frequent responses to toceranib and masitinib in cases of canine MCT carrying a JMR mutation (response rate $\geq 60\%$) compared to those with wild-type JMR (response rate $\leq 30\%$) (Hahn *et al.* 2008, London *et al.* 2009). A subsequent small clinical trial confirmed a numerically superior response rate in JMR mutated cases *versus* wild-type and increased response in both groups compared to equivalent groups treated with vinblastine chemotherapy (Weishaar *et al.* 2018). Based on this work some clinicians are personalising treatment to conventional chemotherapy or a tyrosine kinase based upon JMR mutation status (a work example of this concept is shown in Fig 4). It is worth noting, however, that the above-mentioned study failed to demonstrate enhanced survival when treating a mixed group of MCTs with toceranib/prednisolone compared to conventional vinblastine/prednisolone chemotherapy (Weishaar *et al.* 2018). Possible explanations include more rapidly developing resistance to TKI's than conventional chemotherapy and improved survival of mutation-positive patients, but from a lower baseline leading to overall equivalence in survival. The small (in statistical terms) numbers of mutation-positive tumours in each group and their unequal distribution also adds to the uncertainty. This study therefore serves to highlight some of the issues around evaluating and confirming the validity of precision medicine approaches which are discussed in section "Challenges on the road from molecular pathology to precision medicine."

Less frequent uses of molecular pathology – Diagnosis of chronic myeloid leukaemia and transmissible venereal tumour

Chronic myeloid leukaemia (CML) is rare in pet species and historically diagnosis has been difficult due to a lack of definitive diagnostic tools. A cytogenetic approach has been developed for diagnosis of CML in dogs based on the detection of a fusion gene similar to that seen in human CML. The "Philadelphia" chromosome is a shortened human chromosome 22 that results from reciprocal exchange of DNA between the long arms of chromo-

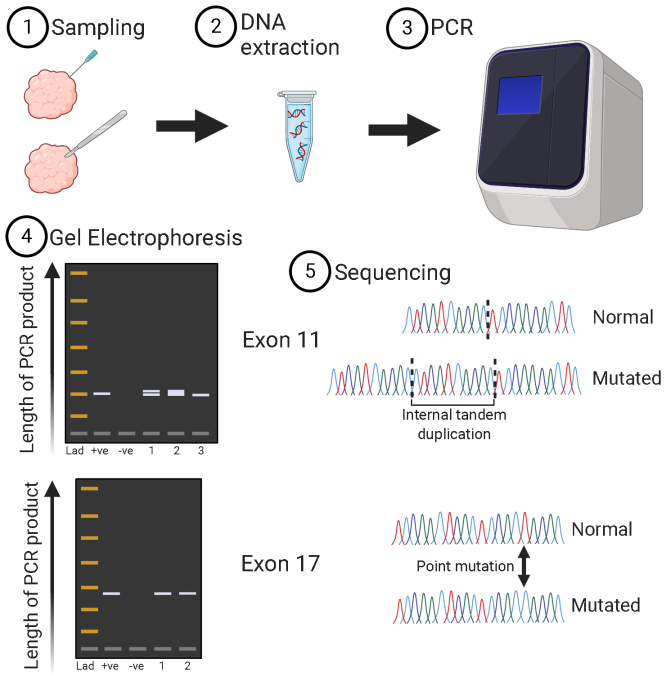

FIG 4. KIT mutational analysis. 1. Samples are collected by FNA or biopsy/excision. 2. DNA is extracted from the tumour cells. 3. PCR is performed using standard primer sets which flank areas of common mutations in exons 11 and 17. 4. PCR products undergo gel electrophoresis. In the exon 11 example the +ve control is the normal exon 11 sequence. In lane 1, two PCR products can be seen therefore presence of an ITD mutation is considered very likely. In lane 2, a thick band is shown making it impossible to determine whether an ITD is present; sequencing is necessary to resolve this uncertainty. In lane 3, the amplicon is the same size as the +ve control hence a normal wildtype exon 11 is likely present. In the Exon 17 example the bands in lane 1 and 2 are similar to the positive control, however, exon 17 mutations are typically point mutations and therefore are unlikely to be identified by this method and thus sequencing is more important for confirmation. 5. Sequencing results; (each peak indicates one DNA base and the colour indicates the base type) show the difference between point and ITD mutations. Created with BioRender.com.

somes human 9 and 22 due to the fusion of *BCR* and *ABL* to form a chimeric gene. The Bcr-Abl protein has constitutively activated tyrosine phosphokinase activity, which is thought to be the main oncogenic driver in >90% of human CML cases, 20% of acute lymphoblastic leukaemia cases and 2% of acute myeloid leukaemia (AML) cases (Kurzrock *et al.* 2003). Identification of the Bcr-Abl fusion led to the first rationally designed drug, imatinib, and thus a new paradigm in cancer treatment (Kurzrock *et al.* 2003).

Fluorescent hybridisation in situ has been used to identify a canine fusion protein equivalent to the "Philadelphia" chromosome. In dogs, this fusion occurs between chromosome 9 and 26 (Breen & Modiano 2008) and has been reported in small numbers of cases of CML, AML and chronic myelomonocytic leukaemia (Breen & Modiano 2008, Cruz Cardona *et al.* 2011, Figueiredo *et al.* 2012, Culver *et al.* 2013, Pérez *et al.* 2013, Marino *et al.* 2017). Presence of this fusion gene allows a diagnosis of CML to be made with greater confidence and it may also guide clinicians towards treatment with imatinib, although this is yet to be systemically evaluated in canine CML.

Fusion genes that mimic those seen in human cancers have been reported in a number of other canine cancers including B cell lymphoma and glioma (Breen & Modiano 2008, Ulvé *et al.* 2017) and this is an area of ongoing investigation.

Canine transmissible venereal tumour (CTVT) is a neoplasm that is naturally transmitted as an allograft between dogs through coitus, licking, biting and sniffing tumour affected areas. Although the origin of CTVT cells has not been well defined, immunohistochemical studies suggest a histiocytic lineage. Clinically a distinction from other round cell tumours, such as MCT, amelanotic melanoma, lymphoma and other poorly differentiated carcinomas, may be necessary in cases where cytological, histopathological and immunohistochemical findings are ambiguous and in dogs with extragenital tumours. CTVT cells have a unique molecular characteristic that can help distinguishing them from somatic cells or other cancers, which is the presence of a long interspersed nuclear element (LINE-1) insertion near the *c-myc* oncogene (LINE-*c-myc*). This rearrangement has been used as a diagnostic tool when detected using *in situ* and conventional PCR techniques (Setthawongsin *et al.* 2016, Castro *et al.* 2017). The LINE-*c-myc* assay requires small amounts of DNA and has shown to improve the diagnostic accuracy of CTVT when compared to standard methodologies. This assay can also be used to monitor response to chemotherapy, especially in dogs where a bulk of fibrous tissue is present after cancer regression. Despite showing promising results, the assay is not widely available and when used, interpretation of the results should be made in conjunction with cytology and/or histopathology.

EMERGING AND FUTURE MOLECULAR PATHOLOGY APPROACHES

Researchers are rapidly expanding the knowledge base for molecular pathology in veterinary medicine. In this section, we highlight some of the potential clinical uses as this research bears fruit.

Genotyping for prediction of cancer risk

Mutations in the tumour suppressor genes *BRCA1* and *BRCA2* are reported in a number of human cancers including breast cancer. As is typical among tumour suppressor genes a variety of mutations/polymorphisms have been reported to lead to deleterious effects with respect to normal function. DNA coding changes leading to reduced or absent function are associated with a large increase in breast and ovarian cancer risk in affected individuals. Moreover, some hereditary *BRCA1* mutations are associated with early onset aggressive triple negative breast cancer. Due to its clinical significance, testing for *BRCA* mutations has become the prototypical example of genetic risk factor screening in humans with relevant familial or ethnic risk factors.

In the veterinary context, breed has been identified as a significant risk factor for the development of numerous cancer types in dogs and cats and it is assumed that genetic polymorphisms underlie the differential risk between breeds. An example

that has gained significant research attention is histiocytic sarcoma in Bernese Mountain Dogs; in this breed the lifetime risk for developing histiocytic sarcoma ranges from 15 to 25%. A genome-wide association study (GWAS) identified a risk allele on the canine chromosome (CFA) 11 and subsequent analyses showed recurrent genomic imbalances including deletions in the tumour suppressor genes CDKN2A/B, RB1 and PTEN (Hedan *et al.* 2011, Shearin *et al.* 2012).

Another example is the predisposition of Swedish English Springer Spaniels to mammary carcinomas; an increased risk has been demonstrated in individuals carrying polymorphisms in the *BCRA1/2* and oestrogen receptor 1 (*ESR1*) genes among others (Rivera *et al.* 2009, Melin *et al.* 2016). More recently, MCTs in Retriever breeds have been a focus of work in this area (Arendt *et al.* 2015, Hayward *et al.* 2016, Biasoli *et al.* 2019). Several polymorphisms associated with increased MCT risk have been identified using GWAS; some are shared between the breeds and therefore worthy of evaluation in a wide group of breeds, but some are unique to different populations. Of interest, each of the affected genes is involved in cell to cell or cell to extracellular matrix interactions (Arendt *et al.* 2015, Hayward *et al.* 2016, Biasoli *et al.* 2019), possibly indicating the value of a more general investigation of this area for MCT.

GWAS has also been used to study the genetic risk factors involved in osteosarcoma across three breeds: Greyhounds, Rottweilers and Irish Wolfhounds. The study identified 33 different inherited risk loci that could explain 55 to 85% of the phenotype variance in each breed with the strongest association found in the Greyhound locus located in the regulatory variants near CDKN2A/B gene, a key regulator of tissue proliferation and senescence (Karlsson *et al.* 2013).

Other studies using GWAS with the aim of elucidating the molecular pathogenesis of haemangiosarcoma, digital squamous cell carcinoma and lymphoma are also available (Tonomura *et al.* 2015). With further work to validate these types of findings, it may be possible to start to estimate the impact of genotype on the risk of particular cancers in individual animals or ahead of breeding decisions.

Biomarkers to predict risk of malignant behaviours and prognosis

History, physical examination, histopathological diagnosis and assessment/grading, and staging are currently used in the clinic to predict the risk of aggressive cancer behaviour. However, even after the application of all of these tools significant uncertainty is often present as to the most appropriate treatment approach, especially with regard to whether systemic therapy is indicated.

Work on various human cancers has demonstrated that genomics/expression profiling can be used to predict metastatic risk (Harris & McCormick 2010). Veterinary researchers have begun to investigate this field and early reports have proposed that assessing expression of different panels of genes may be useful in predicting metastatic propensity in canine MCT and melanoma (Blacklock *et al.* 2018, Blacklock *et al.* 2019) and prognosis in canine lymphoma (Frantz *et al.* 2013, Richards *et al.* 2013).

Identification of novel biomarkers and therapeutic targets to predict ideal therapy choices

In human oncology, the identification of novel biomarkers and drug targets has become a focus of research in cancer therapeutics. Numerous druggable targets have been identified with significant interest in inhibition of kinases; a 2013 review reported almost 130 kinase inhibitor compounds for cancer in clinical trials predominantly focusing on targets familiar to veterinary oncology including VEGF/VEGFR, PI3 kinase, HER2, mTOR, EGFR, MET, PDGF/PDGFR and KIT (Workman *et al.* 2013).

Looking beyond kinases, a recent study reported that over 550 cancer driver mutations have been identified across human cancers (Santos *et al.* 2017). This demonstrates the scope for development of biomarkers to guide use of specific inhibitor molecules, but also the scale of the research challenge. The improved bioinformatic tools supporting genomic investigations in veterinary medicine combined with the genetic similarities between dogs, humans and their respective cancers will no doubt allow use of this information to help identify similar targets in canine cancer. It may then be possible to develop or repurpose drugs for veterinary cancers bearing relevant mutations. The previously discussed *BRAF* mutation in urothelial carcinoma may turn out to be an example of the progression from novel diagnostic biomarker to therapeutic target. Early analysis of an ongoing study evaluating a *BRAF* inhibitor in *BRAF* mutation-positive UC has yielded promising results with higher than previously reported response rates for this tumour type (Wiley *et al.* 2019).

Moving beyond targeting single candidate genes/proteins there is the potential to develop larger scale non-candidate gene approaches. In such approaches rather than assessing a single or small group of genes the whole genome and/or transcriptome would be assessed together to try to identify both germline and somatic variations. These data could then be analysed to identify genotypic and / or transcriptomic features indicating susceptibility of the tumour and tolerance of the patient to a wide range of pharmaceuticals. This approach has the advantage that the potential for efficacy of a broad range of candidates can be assessed at once. Mutations that are individually of great clinical significance are thought to be much rarer than those causing more minor effects. Therefore, a second benefit of this type of approach is that it in principle it could incorporate the summation effects of a number variations (polymorphisms or mutations) each of which may be individually little significance, but which taken together may have a clinically relevant effect. Such approaches have been shown to hold promise in human oncology (Von Hoff *et al.* 2010, Tsimberidou *et al.* 2012).

Beside druggable mutations another type of target that could be identified using next generation genomic approaches are targetable neo-antigens. Mutated proteins are common in many cancers and it is believed that mutated proteins are often immunogenic because they have been produced after central immunologic selection (Wang & Wang 2017). Consistent with these observations there is now a recognised association between mutational burden, intra-tumoural immune infiltrates and survival in certain cancers (Rooney *et al.* 2015). Moreover, as these neoantigens are unique to the tumour it is conceptually appealing

to immunologically target cells bearing them and spare normal tissue. Initially, genomic data could be used to detect genes coding for mutated proteins. Subsequently, antigenicity can be predicted using a number of bioinformatic tools and confirmed via experiments assessing activation of patient lymphocytes exposed to these antigens (Kiyotani *et al.* 2018). Combining such data with emerging therapies such as checkpoint inhibitors and chimeric antigen receptor T cells and engineered antibodies such as Bi-specific T-cell engager may allow production of powerful personalised therapies.

Liquid biopsy to reduce morbidity during diagnosis, and facilitate serial monitoring for cancer, and for precision medicine

"Liquid biopsy" is one the more exciting prospects offered by molecular pathology. Cancers shed cells (so called circulating tumour cells; CTCs) and cell components (cell free nucleic acids) into bodily fluids; it is now well established that such genetic material can be isolated and evaluated. The possible clinical uses of these "liquid biopsies" are numerous and include: diagnosis of cancer via DNA mutations or epigenetic markers (such as DNA methylation patterns) on circulating tumour DNA, monitoring of disease burden with greater sensitivity than the current "anatomic" methods (thus allowing earlier detection of relapse), identification of tissue origin of certain cancers, stratification/prognostication and therapeutic efficacy prediction (Blackhall *et al.* 2018, Stewart & Tsui 2018, Chen *et al.* 2020).

The low specificity of health screening patients for cancer and the cost and morbidity associated with biopsy and serially staging of cancers is a significant barrier to early diagnosis of cancer and monitoring of cancer progression throughout its course. A liquid biopsy approach using serial blood sampling can reduced these problems and facilitate diagnosis long before one would have been made via the conventional route (Chen *et al.* 2020). After diagnosis, serial evaluation may allow for early detection of relapse and monitoring of genetic evolution of cancer via during a cancer process (Stewart & Tsui 2018) and individualised data-guided treatment throughout the disease course.

So far liquid biopsy has been used predominantly as a diagnostic and prognostic tool in veterinary oncology. One example already in diagnostic use is the previously mentioned BRAF mutation for the detection of UC. A recent advance of this approach has been the demonstration that mutated BRAF can be detected in blood samples from these patients (Tagawa *et al.* 2020). A prognostic example is PARR in combination with quantitative PCR for monitoring residual disease burden in canine lymphoma patients. This work showed an association between lack of residual detectable clonal lymphocyte receptor DNA after chemotherapy and improved outcome in canine lymphoma (Sato *et al.* 2011). Moreover, a return of measurable receptor DNA precedes clinical relapse (Sato *et al.* 2011, Sato *et al.* 2016). There are now numerous reports of mutations that could be developed as diagnostic or prognostic assay for different cancer types in veterinary species.

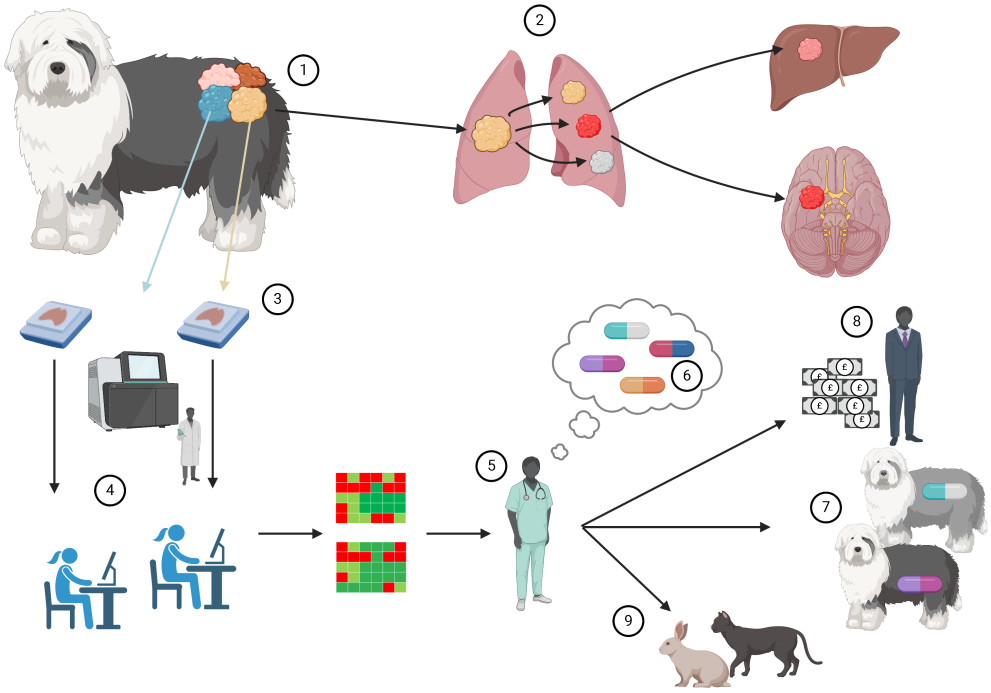

FIG 5. Challenges to overcome on the road to precision medicine. 1. Tumour heterogeneity means that samples may not be representative. 2. Tumour evolution means that lethal clones may not be represented within the primary tumour. 3. Sampling and fixation methods may lead to artefact. 4. Increasingly complex techniques (such as NGS) require significant investment in equipment, training and bioinformatician expertise. All techniques would benefit from across lab standardisation and quality assurance 5. Clinicians must understand the strengths and weakness of data produced and how to act upon it. 6. Therapeutics suitable to target the identified aberrations/mutations are required and their benefit proven. 7. Pharmacogenomic research is needed to identify considerations around individual pharmacokinetics for individual patient/treatment combinations. 8. Meeting these challenges will come at a cost which needs to be affordable to the client. 9. The case for precision medicine in less numerous species and those less frequently affected by cancer is harder to make from an economic perspective. Created with BioRender.com.

CHALLENGES ON THE ROAD FROM MOLECULAR PATHOLOGY TO PRECISION MEDICINE

The approaches described above are conceptually straightforward, but there are significant practical issues that will need to be overcome in order to leverage the most benefit from each of them (summarised in Fig 5). In the final section of this article, we discuss the more significant of these issues.

Sample collection, technical standardisation and quality assurance

Problems during sampling, delays in fixation and fixative choices can have a significant impact on subsequent transcriptomic and proteomic analysis. Conversely, genomic DNA is relatively resistant to the post sampling environment. A range of factors may influence stability of molecules within tumour tissue including: temperature changes, changes in oxygen concentration in tissues during anaesthesia and after excision and exposure to the atmosphere, effects of tissue handling, choice of fixative and time to and rate of fixation (Liotta *et al*. 2009, Arima *et al*. 2016). For example, RNAase inhibitors (*e.g*. RNAlater) have been developed which effectively stabilise the transcriptome if used soon after sampling (Shabihkhani *et al*. 2014). Conversely, formalin fixation leads to fragmentation of nucleic acid chains and base mutations thus reducing the sensitivity and accuracy of nucleic acid-based assays (Greytak *et al*. 2015). Therefore, ideally a standardised approach that preserves sample quality should be developed for collecting and fixing samples.

Beyond preservation it is also vital that sufficient quantities of tumour DNA (relative to normal DNA and methodologic limits on sensitivity) are collected within a sample. The nature of the clinical situation therefore has the potential to significantly impact upon test performance. For example: tissue biopsies collect more cells and are therefore likely to be accurate than fine needle aspirates for assessing whether lymph nodes contain early metastatic disease. Moreover, intra-tumoural heterogeneity combined with low volume samples will lead to a greater risk of producing non-representative results.

Beyond the samples themselves, the robustness of the assays will also influence results. The technology available for genomic evaluations is rapidly developing both in terms of technical capability and breadth of providers. The authors therefore have not included discussion of the strengths and weaknesses of particular methodologies, but cost, sensitivity, specificity, precision, accuracy and reproducibility will be relevant to the feasibility of bringing assays into clinical use. A key consideration is that most of the tests are currently only offered by a handful of laboratories. Typically laboratories are therefore working to an in-house rather than a standardised quality assured methodology; there is therefore potential for significant variation in assay performance between laboratories. This can make it difficult for the user to know how precisely the research data can be applied to the clinical results they are considering.

Generation of data in a clinically relevant time frame

One of the challenges in a precision medicine approach is the generation and analysis of the data in a clinically relevant time frame. Next generation sequencing data is starting to come into routine clinical use in human oncology. A precision medicine approach leveraging data in the quantities produced by NGS requires specialist informatics skills and significant computer processing power to produce results in a form usable by clinicians. Two studies have evaluated such approaches in the veterinary context and found that results could be provided in 5 to 10 days, if appropriate infrastructure is available (Monks *et al*. 2013, Paoloni *et al*. 2014). It is feasible that this time frame could be reduced further by using the new generation of miniaturised sequencers (such as MinION, Oxford Nanopore, UK) to produce data in the clinical setting before transferring the data for analysis at a centralised external facility. Whilst demonstrating feasibility these studies highlight the need for dedicated infrastructure (both human expertise and technological) in order to support such a responsive service and inevitably this infrastructure will add to the cost of treatment and require a certain case volume in order to be viable. The ongoing Canine Hemangiosarcoma Molecular Profiling (CHAMP) (Katogiritis & Khanna 2019) study will further test the feasibility of these strategies in veterinary medicine.

Understanding how to make genomic data actionable

With the technology now available, it is relatively facile to produce large quantities of high-quality genomic data from well prepared clinical samples. However, accurately interpreting this data and making it usable in a wide range of clinical settings carries significant challenges and prior knowledge requirements.

The first challenge to actioning this information is the need to for research to support the relevance (or otherwise) of particular genomic variations. So far evaluations of non-candidate personalised medicine approaches have often relied on comparisons with human data. The applicability of much of this data in the canine setting is uncertain at this stage. A second more general issue is that genetic mutations and transcriptomic variations are not always predictive of altered protein or metabolic function and may be contextually dependent on tumour type in their effects. Epigenomics, proteomics and metabolomics are rapidly developing fields and a full understanding of the molecular pathology of a particular disease will only be realised when data from each of the fields can be integrated with each other and with clinical data.

Even once there is a full suite of well-developed technologies and data available tumour heterogeneity will continue to represent a considerable challenge. A passing microscopic evaluation dispels any notion that tumours are homogenous blobs. Rather tumours are a complex ecosystem comprised for cancer cells evolving within a variety of evolutionary niches shaped by factors such as tumour hypoxia and the hosts immune system. Consequently, heterogeneity is present both within and between constituent cell sub-populations contained within a tumour (Liotta

et al. 2009, Altschuler & Wu 2010, Lawrence *et al*. 2014, Andor *et al*. 2016, Cyll *et al*. 2017). This produces a number of challenges for the successful pursuit of molecular pathology and even more so for precision medicine.

Genomic techniques use minute amounts of material (milligrams or less) and this small sample size leads to an increase risk of assay failure due to inadequate numbers of tumour cells and excessive necrosis. In two of the aforementioned studies, these problems were associated with a sample quality failure rate of around 25% (Monks *et al*. 2013, Paoloni *et al*. 2014). A further biological issue alluded to above is that due to heterogeneity, the chances of a single sample being unrepresentative of the genetic diversity of the cancer as a whole (and the lethal clones specifically) is high (Cyll *et al*. 2017). It is therefore easy to imagine that selecting a part of a tumour at random may lead to non-representative results. One example of how this might alter interpretation is that relevant tumour DNA may not be detected if present below the limit of detection of assay in the particular sample submitted (*e.g*. if a relevant mutation is minimally present in sample tested). To overcome this issue, it is logical to sample areas of the tumour that appear to be of highest histological grade, but there is at least one report of lethal clones arising from apparently lower grade parts of a tumour (in that case prostatic carcinoma) (Haffner *et al*. 2013); this highlights that it cannot be assumed that focusing on the areas appearing to be most aggressive will lead to predictive results. Options for mitigating heterogeneity include laser capture microdissection to ensure adequate tumour cell content or sampling from multiple areas of a tissue sample. Certainly, increasing spatial distribution of sampling may help mitigate against intra-tumoural heterogeneity. It is, however, unfortunately not an infallible solution because intra-tumoural heterogeneity rather than being an entirely random event both indicates and drives a process of evolution. Therefore the presence of sub-clonal populations makes it unlikely that one can evaluate a tumour as the average of its parts rather than as individual entities each of whom may have their own behavioural traits, as demonstrated by the prostatic carcinoma example above [and nicely explained in a review by (Altschuler & Wu 2010)].

Finally, the complexity of these challenges is heightened further by the knowledge that metastatic and primary tumours may not have the same driver mutations due to constant evolution within tumours and their metastasis (McGranahan & Swanton 2015). Contextual nuancing of the effect of a particular mutation by interactions between tumour and microenvironment can add further complexity (McGranahan & Swanton 2015). Hence, mutations in the primary tumour may often not be useful in guiding life-saving anti-metastatic treatment as their driver mutations may not be the same as those in the lethal clone. In this regard monitoring through liquid biopsy (especially of CTCs) provides an appealing opportunity to evaluate disparate parts of a cancer process and assess its evolution over the course of disease (Ulz *et al*. 2017).

Considering the patient side there are also challenges to consider. Hitherto very few significant pharmacogenomic variations relevant to cancer treatments have been identified in pet species. It is likely that the number of polymorphisms leading to large pharmacokinetic effects is very limited and that much of the phenotypic variation is due to the combinatorial pharmacogenomic effects of multiple polymorphisms, thus making them more difficult to identify without assessing a very large population of pets (which may be prohibitively expensive). Consequently, it is more likely that we will be able to identify groups of patients that have markedly greater or lower tolerance of a particular agent than tailor our dosing of an anti-cancer therapy to each individual patient.

Challenges to the application of molecular pathology are also present in the preventative setting. Identification of risk factor genes may help efforts to reduce breed predispositions if breeders and breed societies can be successfully engaged, but on a per patient basis knowing how to act on that information is more difficult. Considerable effort goes into genetic counselling of humans with polymorphisms associated with increase cancer risk (such as BRCA1) in order to help them make the best preventative decisions. Many owners already accept breed-related disease as part of the experience of owning a particular breed and given the cost constraints in the veterinary setting they may not wish to know that their pet is at increased risk of a particular cancer. Indeed, as breeders are predominantly selling a breed-phenotype it may actively run against their financial interest to make owners aware of disease risk factors carried within their breeding line.

Need for robustly validated clinical evidence

Genetic heterogeneity at the tumour, individual and population level all speak to the necessity of a robust evidence base to support use of molecular pathology for precision medicine. The relevance of diagnostic and prognostic markers need to be confirmed in validation studies and ultimately, the benefit of precision medicine treatments guided by such markers will need to be proven; ideally in randomised clinical trials (Webster *et al*. 2011).

A criticism of some recent trials of new agents in human oncology is that they have been overpowered to identify a difference that was statistically significant, but of minimal clinical relevance (Fojo *et al*. 2014). In veterinary medicine financial constraints mean that underpowering is more likely to be a problem, but philosophical consideration should be given to trial design such that studies have well-defined inclusion criteria and are powered correctly to produce clinically meaningful outcomes. In the context of much smaller veterinary trials, a recent modelling study using realistic estimates of effect sizes found that small trials with P values near P = 0.05 frequently yield false positive results (estimated at 54% in studies of 20 subjects per groups and P = 0.05 and still 33% in studies of 50 subjects per group) (Van Calster *et al*. 2018) and therefore evaluation of reproduction of small scale trials is of great importance.

Commercial challenges

An important consideration for the veterinary oncology community and industry is whether human style precision medicine with individualised treatments tailored to the genetic profile of the patient and tumour are likely to be feasible as a general principle rather than in relation to only the common clinical problems and mutations. The answers may differ in relation to the

development of diagnostic tests or enhancing use of therapeutics already available *versus* development of new drugs.

Optimal treatment of aggressive cancer is expensive and often involves a degree of morbidity for the patient in order to allow a diagnosis and full assessment of disease burden. In time, molecular pathology will provide a variety of tests that will allow a clinical diagnosis to be made at reduced cost and morbidity in many cancers. Moreover, it may be possible to monitor disease state by serial evaluation of biomarkers thus reducing the cost and morbidity whilst potentially facilitating earlier detection of relapse. If these evaluations can be made using relatively non-invasive tests such as liquid biopsy the savings made by avoiding traditional biopsy and staging procedures may provide financial headroom for increased spending on treatment. Or even more optimistically guide clinicians to withhold medical therapy altogether when the data suggests it is unlikely to be beneficial.

Conversely, in regard to the development of novel therapies, the experiences of research in human oncology should give veterinary oncology researchers pause for thought. A number of targeted agents have been licensed for human use in recent years, yet less than half of them meet modest efficacy standards developed by expert working groups (Kumar *et al*. 2016). Moreover, unsurprisingly given the costs of developing new drugs, industry has prioritised more appealing targets leading to duplication of products for more common indications and a dearth for others (Fojo *et al*. 2014). Similar duplication is the norm in the veterinary ectoparasiticide market and has already occurred in veterinary oncology in the case of tyrosine kinase inhibitors masitinib and toceranib. Given the more limited resources available for veterinary product development a collaborative approach between providers would likely lead to broader provision, but the commercial interests at play may make this difficult to achieve. One route to reducing the cost of development of novel drugs may be to repurpose drugs which are no longer being developed for human use, an approach already used successfully to bring rabacfosadine (Tanovea-CA1, VetDC), for treatment of canine lymphoma, to market in the USA.

Felis catus – the forgotten species?

The available genomic data for cats (and less commonly kept species) lags far behind what is available for dogs and as the vast majority of the oncologic research effort is focussed on dogs, the knowledge gap continues to widen (Katogiritis & Khanna 2019). Moreover, dogs have evolved within a human environment and as such have taken on some traits of human metabolism; as a result of which their cancers may be more analogous to ours than to cats. Therefore, the benefits of molecular oncology will be brought to dogs much earlier and more broadly than to cats.

CONCLUSIONS

For reasons of practicality and cost traditional microscope-based pathology and imaging-based clinical staging are likely to remain the most important diagnostic tool in veterinary cancer care for some time to come. However, molecular pathology will increasingly augment this information and over time may start to supplant aspects of it by allowing more certain diagnosis and improved prognostication with lower morbidity. Beyond that, the tools to support precision medicine are increasingly available, but there is much data to be generated and significant challenges to be overcome before the full potential of precision medicine for veterinary cancer patients can be realised.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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