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CANCER-INDUCED MUSCULOSKELETAL DISEASES (E KELLER AND J STERLING, SECTION EDITORS)

Osteosarcoma in the Post Genome Era: Preclinical Models and Approaches to Identify Tractable Therapeutic Targets

Wilson Castillo-Tandazo[1,2] · Anthony J. Mutsaers[3] · Carl R. Walkley[1,2,4]

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Abstract

Purpose of Review Osteosarcoma (OS) is the most common cancer of bone, yet is classified as a rare cancer. Treatment and outcomes for OS have not substantively changed in several decades. While the decoding of the OS genome greatly advanced the understanding of the mutational landscape of OS, immediately actionable therapeutic targets were not apparent. Here we describe recent preclinical models that can be leveraged to identify, test, and prioritize therapeutic candidates.

Recent Findings The generation of multiple high fidelity murine models of OS, the spontaneous disease that arises in pet dogs, and the establishment of a diverse collection of patient-derived OS xenografts provide a robust preclinical platform for OS. These models enable evidence to be accumulated across multiple stages of preclinical evaluation. Chemical and genetic screening has identified therapeutic targets, often demonstrating cross species activity. Clinical trials in both PDX models and in canine OS have effectively tested new therapies for prioritization.

Summary Improving clinical outcomes in OS has proven elusive. The integrated target discovery and testing possible through a cross species platform provides validation of a putative target and may enable the rigorous evaluation of new therapies in models where endpoints can be rapidly assessed.

Keywords Osteosarcoma · Mouse models · Patient-derived xenograft · Canine OS, sarcoma

Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents aged 10–20 years. It is commonly located in the metaphyseal growth plates of the long bones of the extremities [1]. Histologically, OS is characterized by the production of bone stroma by malignant osteoblastic lineage cells, which can be further classified into osteoblastic, chondroblastic, and fibroblastic subtypes [2]. While histological subtypes are described, current standard-of-care treatment and outcomes are independent of the subtype ascribed. Numerous risk factors for OS development have been determined including rapid bone turnover and growth, exposure to radiation, and genetic diseases such as Li-Fraumeni syndrome, hereditary retinoblastoma, Rothmund-Thomson syndrome (RTS), RAPADILINO syndrome, Werner's syndrome, and Bloom's syndrome [3]. The vast majority of OS occurs sporadically. OS is characterized by complex genomic structures and rearrangements as well as high interpatient heterogeneity, making the identification of broadly applicable therapeutically actionable pathways a challenge. Patients with non-metastatic OS have a 5-year survival rate of 60–70%, which is dramatically reduced to 20–30% when metastases develop [4]. Despite the stabilization of survival rates over the last 30 years, the treatment of OS still relies on surgery and combination chemotherapy, with very few

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✉ Carl R. Walkley
cwalkley@svi.edu.au

✉ Anthony J. Mutsaers
mutsaers@uoguelph.ca

[1] St. Vincent's Institute, 9 Princes St, Fitzroy, VIC 3065, Australia

[2] Department of Medicine, St. Vincent's Hospital, University of Melbourne, Fitzroy, VIC 3065, Australia

[3] Department of Biomedical Sciences, Ontario Veterinary College, Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Canada

[4] Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC 3000, Australia

modifications in several decades [5]. This has led to a plateau in survival rates, particularly in metastatic OS, but no significant improvements in therapeutic response have been achieved since the introduction and optimization of chemotherapy regimens.

Whole genome and exome sequencing (WGS/WES) studies have characterized the genomic landscape of OS, defining it as strikingly complex [6, 17, 18••, 19, 20]. OS tumors are aneuploid and have high rates of chromosomal aberrations, including chromothripsis [20] and the presence of hypermutable regions known as "kataegis" [17]. *TP53* is the most frequently mutated gene in OS and has a long-known association with OS due to the overrepresentation of OS in the spectrum of cancers affecting Li-Fraumeni syndrome patients. The sequencing of the OS genome reported > 90% TP53 mutations in one allele and > 80% in both alleles [17], a finding replicated across studies and cohorts [6, 21–24]. Interestingly, there is an apparent preference in OS for null or loss of protein expression mutations in *TP53* due to the inversion of intron 1. The reason for the preference for inactivating/null mutations, rather than the more prevalent hotspot point mutations seen in many cancers, has not been resolved. Other genes recurrently mutated in sporadic OS include *RB1*, *ATRX*, and *DLG2* [17, 18••]. In Rothmund-Thomson syndrome, a rare autosomal recessive disorder caused by mutations in the *RECQL4* gene, OS is reported in 32% of patients and remains among the top causes of mortality in these patients [25]. Despite the OS predisposition in RTS, mutations in *RECQL4* are not found in sporadic OS for at present unknown reasons.

Our understanding of the genomic landscape of OS is approaching saturation. This information has principally derived from a combination of exome, whole genome, and RNA sequencing studies, with some limited additional insight from genome-wide association studies. This has provided a comprehensive list of genes that can contribute to OS, but has not identified readily actionable therapeutic targets that would be broadly applicable. As a result, additional approaches are required to identify and test therapeutic vulnerabilities and then triage these to prioritize clinical testing. Three models using primary tumor material have been developed and applied: genetically engineered murine models, patient-derived primary tumor material (both in cell culture and as xenografts), and the spontaneous disease that arises in large breed dogs (Fig. 1). Here we will review how these models have been utilized to progress therapeutic target identification and development in OS. (Table 1).

Preclinical Models: Genetically Engineered Murine Models of OS

Initial high fidelity, autochthonous murine models of OS were generated over a decade ago, based on the known genetics of familial cancer syndromes predisposed to OS. These models utilized the loss of *p53* and *Rb* in cells of the osteoblastic lineage, allowing the development of OS with full penetrance and high metastatic rates [26–30]. Extensive characterization of these models at the histologic, transcriptomic, genomic, and most recently genetic/therapeutic vulnerabilities has established that these models are faithful representations of OS as it occurs in humans. The murine models demonstrated an absolute requirement for mutation of *p53*, a finding consistent with the near universal mutation of *TP53* in human OS. In the mouse models, the loss of *Rb1* alone was not sufficient to initiate OS, but when lost with *p53* was a potent accelerating event. Interestingly, the genetic cooperativity between p53/Rb loss at accelerating OS is apparent only within the committed osteoblast populations (as marked by *Osx1*-Cre expression), as when p53/Rb are concurrently deleted in more immature populations (marked by *Prx1*-Cre), the rate of OS drops and undifferentiated sarcomas predominate [27, 31]. Retrospectively, this may not be surprising given the shared mutational spectrum of the undifferentiated sarcoma genome and OS, suggestive of a significant contribution from the cell of origin to the ultimate tumor that arises [32].

Additional models have recently been described. Using a range of different Cre drivers, Quist et al. were able to demonstrate that OS can arise from across a differentiated spectrum of cells within the osteoblastic lineage. Strikingly, even cells within the committed osteoblast populations were able to efficiently initiate OS in vivo [29]. These findings, together with data generated in a range of other independent models using additional Cre drivers, are consistent with the cell of origin of OS coming from within the osteoblastic lineage [33]. Several models have recently been described using hot spot mutations of p53, rather than the null alleles used previously [34, 35]. One study found that while disease latency was comparable between the different p53 alleles, the metastatic frequency was higher in a p53R172H point mutant model (53% of animals with metastatic dissemination) compared with the *p53* null allele (25% of animals) [35]. We previously reported metastatic rates of 50% in *Osx1*-Cre $p53^{fl/fl}$ mice and up to > 80% in *Osx1*-Cre TRE-p53.1224 (an osteoblast-restricted shRNA directed against endogenous p53) [28], suggesting that there may be difference across cohorts (e.g., mouse strain, overall health status) and animal facilities. A separate study also applied a p53 point mutant allele and *Osx1*-Cre in a mutagenic model using sleeping beauty transposase [31, 34]. Given the mutational preference in human OS for null alleles, often as a result of inversions of the first intron which have been found in both sporadic OS and Li-Fraumeni patients, the use of genetically modified null alleles would appear to more closely mirror the human disease. A detailed comparison between murine OS tumors derived from null alleles to those harboring p53 point mutations would be warranted to resolve if there are fundamental differences generated by the p53 mutation type applied. This is particularly

Table 1 Summary of the most recent targeted therapeutics identified for osteosarcoma

Drug/molecule	Pathway involved	Mechanism/effect	Model tested	Evidence from multiple species?	Reference
Dual PI3K-mTOR inhibitor • GSK2126458 • PKI-587 • BEZ-235	PI3K pathway/mTOR pathway	Dual inhibition of PI3K and mTOR pathways was more potent than PI3K-selective agents on established OS cell lines	Murine primary/met cell lines; human primary cell lines (PDX derived)	Yes; currently in phase II clinical trial (MATCH study: Clinicaltrials.gov ID NCT03213678)	[6, 7]
BET bromodomain inhibitor • JQ1 • I-BET151 • I-BET762	BET proteins/chromatin	Inhibition of the bromodomain of the BET signaling pathway disrupted the vicious cycle between bone-associated tumors and bone resorption through transcriptional repression of *MYC*, *RUNX1*, or *FOSL1*	Human OS cell lines; mouse cell lines in vitro and in vivo	Yes; currently in phase I clinical trials	[8, 9]
IAP antagonists (SMAC mimetics) • SM-164 • GDC-0152 • LCL161	Apoptosis pathway	Activation of caspases through the inactivation of inhibitors of apoptosis molecules (XIAP and cIAPs)	Murine primary/met cell lines	Not for OS	[10•]
PI3K inhibitors MEK inhibitors	PI3K pathway/MEK pathway	Dual inhibition of PI3K and MEK pathways showed to be effective in solid tumor xenografts carrying KRAS mutations, HER2 amplifications, or PTEN deletions and mutations	Human primary OS cell lines (PDX derived); in vivo PDX	Yes; phase I clinical trials	[11]
HDAC inhibitors • Parabinostat • Romidepsin	Chromatin/transcription factors: Runx2/Wnt/B-catenin pathway	Induce maturation and differentiation of bone through the inhibition of transcription factor Runx2 and regulation of Wnt/B-catenin pathway. When combined with nucleoside analog gemcitabine or topoisomerase inhibitor, doxorubicin showed synergistic effects	Human OS cell lines	Not yet in OS—romidepsin used for soft tissue sarcomas	[11••, 12]
Listeria vaccine expressing *HER2* (ADXS31-164)	HER2 targeting; immune response	Anti-HER2 immune response, T lymphocyte activation, interferon responses; improved survival compared with historical control	Canine phase I clinical trial		[13]
Salmonella encoding IL2 (SalpIL2)	IL2 expression	Recombinant *Salmonella* expressing IL-2 vaccine; delivery of IL-2 to hypoxic tumor	Canine phase I clinical trial		[14]
Viral vector gene therapy e.g., Adenoviral FasL Oncolytic therapy (viral) e.g., (VSV-IFNβ-NIS)	Various FasL; interferon beta and sodium iodide symporter	Stimulators of immune response, tumor lysis; stimulation of inflammation and lymphocyte infiltration into the tumor	Canine clinical trials		[15, 16]

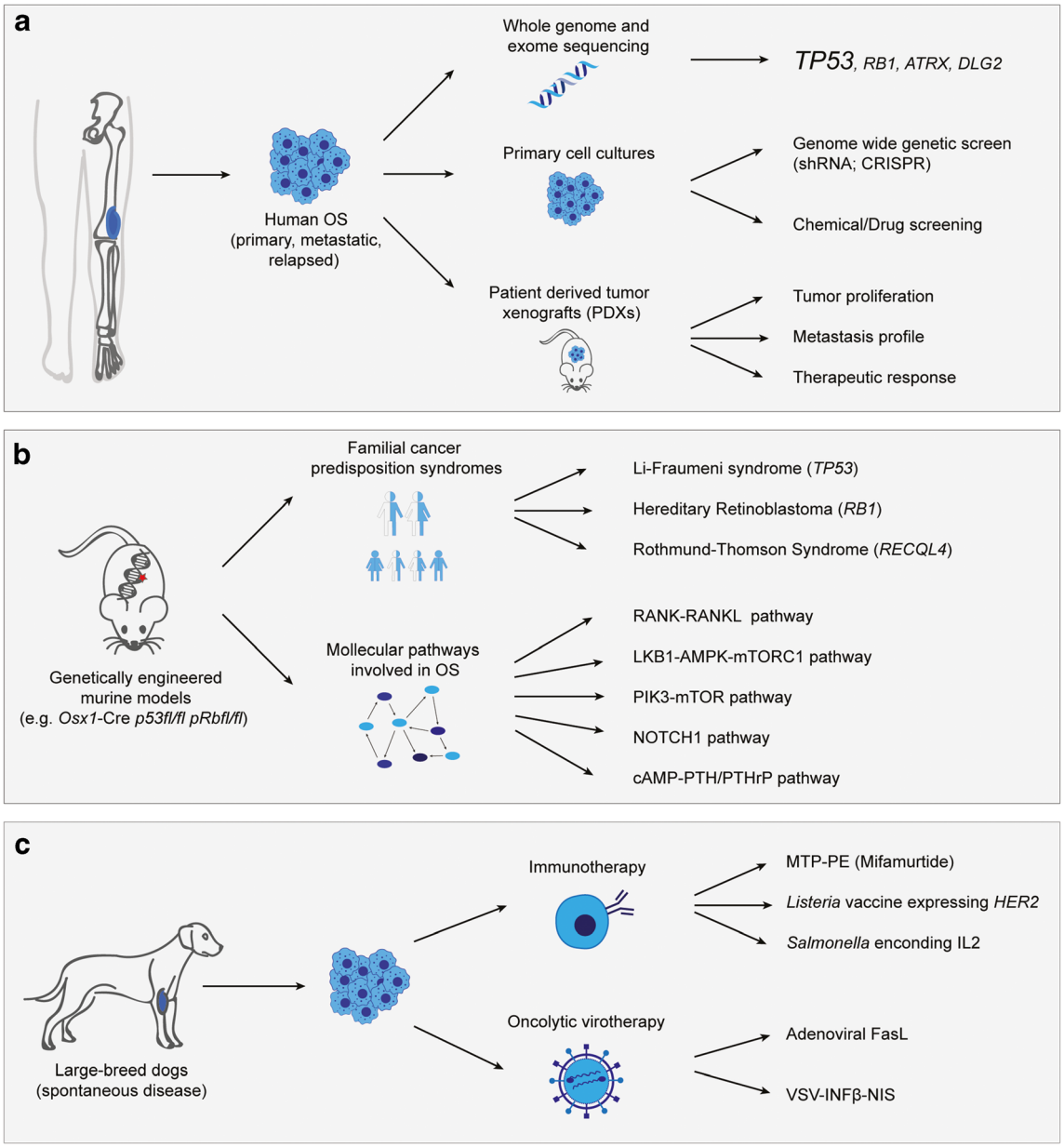

Fig. 1 Different preclinical models and approaches for progressing preclinical targets in osteosarcoma. **a** Human OS samples and primary cells derived from them have been applied to characterize the genomic landscape through both whole genome and exome sequencing. The use of these as primary samples and cell cultures has contributed to the identification of therapeutic vulnerabilities through chemical/drug and genome-wide genetic screens. Additionally, their engraftment in immunodeficient mice (patient-derived tumor xenografts (PDXs)) has been used to study the proliferation, metastatic profile, and therapeutic response of OS in experimental designs mimicking clinical trials. **b** Genetically engineered murine models have been used to understand the high prevalence of OS in several familial cancer predisposition syndromes: Li-Fraumeni syndrome (*TP53*), hereditary retinoblastoma (*RB1*), and Rothmund-Thomson syndrome (*RECQL4*). They have also been applied for target discovery and validation studies to identify and establish evidence for targeting novel molecular pathways, including RANK-RANKL, LKB1-AMPK-mTORC1, PIK3-mTOR, NOTCH1, and cAMP-PTH-PTHrP pathways. **c** Large breed dogs provide an attractive model for OS preclinical development due to their high resemblance to the human disease, the spontaneous development of OS (i.e., non-genetically modified animals), and the advantage of studying OS in an immunocompetent organism that shares the human environment. They have facilitated the development of current treatment approaches and in the testing of novel therapies including immunotherapy and oncolytic virotherapy

important if the gene expression and genetic signatures of these murine models are to be utilized in cross species comparisons and to validate their utility as preclinical platforms [7, 28, 30, 36].

More recently, a number of different models have been described to result in OS in vivo and have been applied to understand the roles of additional genes in OS initiation. In contrast to the OS predisposition in Rothmund-Thomson

Syndrome patients, we and others have failed to observe OS in models based on the deletion of *Recql4*, the described genetic cause of RTS [37, 38]. While the low bone mass of RTS patients can be recapitulated in the murine models, the mice do not develop OS suggesting either species differences or that pathogenic mutations in *RECQL4*, rather than null alleles, need to be assessed before a final conclusion can be drawn regarding the role of RECQL4 in OS initiation. One of the more confounding recent studies reported the development of OS following the expression of activated Notch in the osteoblastic populations [39]. While the analysis provides support for the conclusions reached, the relevance of this model to understanding human OS is questionable given the lack of mutations in *NOTCH1* or any of its ligands in human OS [6, 17, 18••]. Another pathway explored in vivo in murine models is the role of RANKL and its receptor (RANK), a signaling pathway essential for bone homeostasis through the differentiation and activation of osteoclasts [40]. The conditional deletions of RANK in either the osteoblast or osteoclast lineage were assessed using the osteocalcin-driven SV-40^{Tg} (MOTO) background. The authors proposed that deletion of RANK in osteoclasts inhibits OS formation [41]. It is important when interpreting the conclusions to consider that the study used *Mx1*-Cre to induce the conditional deletion of *Rank* allele, and numerous studies have demonstrated that *Mx1*-Cre is not specific to osteoclasts but able to activate gene recombination broadly, including efficiently in hematopoietic and skeletal stem and progenitor cells [42–45]. Han et al. generated a mouse model based on the conditional knockout of *Lkb1* (also known as Stk1) in periosteum-derived *Ctsk*-Cre-expressing cells. LKB1 is a serine/threonine kinase that phosphorylates AMPK, which inactivates mTORC1 in the LKB1-AMPK-mTORC1 pathway [46]. The authors found that loss of *Lkb1* in this model led to OS formation by increasing mTORC1 activity [47•]. Although mutations in *LKB1* are not commonly found in human OS, reduced LKB1 protein expression has been found in 41% of OS patients [48]. This may explain why this pathway is affected in OS and could also serve as a potential therapeutic target.

The development and validation of independent murine models of OS has cemented the central role of mutation of p53 in OS initiation. These models have now been utilized to explore the in vivo genetics of OS and in a range of preclinical target identification approaches. One advantage of the murine models is the ability to isolate and establish treatment-naïve paired primary and metastatic primary cell cultures, enabling a range of complementary experimental approaches. This has recently been matched by the establishment of a number of primary human OS-derived cell cultures. The murine cell lines derived from the *Osx1*-Cre $p53^{fl/fl}$ $pRb^{fl/fl}$ models have proven to be highly useful for pre-clinical screening, enabling both chemical/drug screens and genome scale loss of function studies using both siRNA and shRNA [6, 7]. Independent, orthogonal approaches converged to provide evidence that targeting of the PI3K-mTOR axis was a therapeutic vulnerability in OS [6, 7]. These studies were able to confirm that this was species conserved, utilizing primary early passage cell lines derived from patient-derived xenografts. Supporting the findings of these agnostic chemical-genetic screens, the PI3K-mTOR pathway was also targeted in a transposon-based mutagenesis model of OS [34]. Pathway-specific studies have also been reported. A number of preclinical studies have assessed the efficacy of new generation epigenetic-targeted therapy in OS models. These have demonstrated in vivo activity of small molecule inhibitors of the bromodomain and extra terminal repeat (BET) family [8, 9]. The activity of BET inhibitors was species conserved across both primary mouse and human OS; however, the mechanism of action remains unresolved with discrepancies between that observed in primary tumor-derived samples and from long established human OS cell lines [8, 9]. Small molecule inhibitors targeting cell death–related pathways have also demonstrated efficacy against murine OS-derived cell cultures [10•].

Another pathway that has become increasingly understood through application of the murine OS models is the role of cAMP and parathyroid hormone (PTH)/parathyroid hormone–related protein (PTHrP) and their shared cell surface receptor (PTHR1) in OS biology. These pathways are intimately involved in osteoblast biology and therapeutics targeting this pathway in clinical use for the treatment of low bone mass. The clinically applied agent, PTH [1–7, 17–43], was associated with a high incidence of OS in long-term toxicology studies in rats [49, 50], a finding not reported to date in primate studies of the same agent [51]. OS cells expressed PTHR1 and make autocrine PTHrP, leading to constitutive stimulation of the cAMP/CREB1 pathway [28, 52]. The knock-down of either PTHrP, the most likely ligand for this pathway in OS, or the transcriptional effector of this pathway CREB1, was able to prevent the hyperproliferative transformation of osteoblasts following loss of p53 in vitro, and significantly reduce OS proliferation in vivo [53]. Further studies demonstrated that murine osteoblastic cells do not normally tolerate constitutively elevated levels of cAMP, but can if they become p53 deficient [54••]. This provided a nexus between the near obligate loss of p53 in OS and the ability of these cells to tolerate the sustained early activation of the cAMP pathway. Targeting this pathway with chemical inhibitors of the interaction of Creb1 and its cofactor CBP demonstrated that this is a potentially actionable therapeutic pathway in OS [54••].

Collectively, murine models have provided experimental confirmation of the relative importance of the different genes recurrently mutated in OS. The roles of a number of these still remain to be discerned, such as *ATRX* and *DLG2*, while the murine models have yet to provide further insight into why patients with *RECQL4* mutations are predisposed to OS. The models have proven to be a vital source of primary tumor

material with paired metastatic samples and cell cultures, enabling testing of a range of targeted agents and agnostic screening for new vulnerabilities. The studies, contributed to by many groups, have demonstrated that the murine models are an effective platform for understanding OS genetics and biology and for identification of new therapeutic options. The murine models are complementary to two additional preclinical translational platforms: patient-derived xenografts and the spontaneous OS that arises in domestic large breed dogs.

Patient-Derived Tumor Material and Xenografts

As a tumor type, OS is highlighted by complex genomic changes resulting in intra- and inter-tumor heterogeneity. Coupled with the lack of obviously therapeutically actionable targets, such as recurrently mutated kinases, the primarily pediatric cohort impacted by OS, and the relatively small number of overall patients, the introduction of new therapeutic strategies has been gradual. An increasingly favored approach for preclinical therapeutic testing is through patient-derived tumor xenografts (PDX). This approach takes tumor tissue obtained at the time of diagnosis from patients and engrafts the tumor material into immunodeficient mice. One key aspect of this approach is that the source is primary human material, alleviating the concerns that have mounted regarding the use of long-established OS-derived cell lines, which are subject to selection and drift over many generations in tissue culture [55•]. PDXs can be used to understand a tumor's proliferation, metastasis, and therapeutic response both in vitro and in vivo. This can be particularly useful in patients with heterogeneous cancers and a limited therapeutic spectrum allowing a more personalized clinical and therapeutic approach. Several groups have generated OS PDXs achieving high rates of engraftment and recapitulating the human tumor biology [11••, 56–58]. Furthermore, these models have also been used to determine pathways affected and to test new drugs. By implanting primary and metastatic OS tumors into immunodeficient mice, Loh and colleagues recapitulated the key radiographic and histologic features of the original patient tumors and then utilized these xenografted samples to perform drug screening studies using PDX-expanded and previously established OS cell lines [11••]. The authors found that combination of PI3K/MEK and PI3K/mTOR inhibitors was more effective against OS cells than the individual drugs, consistent with the results from the screening of murine models [11••]. In addition, Histone deacetylase (HDAC) and mTOR inhibitors showed greater efficacy when combined with standard-of-care drugs doxorubicin and gemcitabine [11••]. Such approaches demonstrate the power of primary human PDX models for prioritization of agents for clinical testing. As noted by the authors, these models require a high level of technical and experimental skill to generate reproducible in vivo xenografts, due to the preference for orthotopic intratibial/intrafemoral transplant of the PDX. A separate group reported the establishment of 15 PDXs and characterized them using WGS and RNA sequencing. They found that the primary tumors were relatively stable to the corresponding PDXs. They also tested several drugs that blocked the pathways of known amplified genes. This "genome-matched" therapeutic approach had near 60% tumor growth inhibition in vivo, particularly with tumors presenting *MYC*, *CDK4*, *AURKB*, and *AKT1/2* amplifications [59••]. The discoveries made using PDX models are promising; however, they are still not widely utilized at present due to their recent derivation and a number of additional factors such as difficulties/expense associated with the highly immunocompromised murine models required and the relative difficulty in obtaining primary OS samples. A generic challenge of in vivo PDX models, not limited to OS, is the study of immune-based therapies or tumor-host interactions due to the use of highly immunodeficient mice required as recipients. This can be addressed, to a limited extent currently, by humanization of the hematopoietic system of the murine recipients; however, this remains a challenge and needs to be considered. Extensive ongoing work optimizing the ability of immunodeficient mice to support full human hematopoiesis will eventually overcome this hurdle.

Preclinical Studies: Canine OS

Similar to humans, OS is the most common primary bone tumor in domestic dogs. However, unlike people where OS is a rare cancer type, OS is much more common in large and giant dog breeds. There are ~ 75,000 new OS cases diagnosed in pet dogs per year in the USA, which is approximately 75-fold higher than in humans [60]. Beyond the high incidence, rapid translation of results is further facilitated by the fact that the normal canine lifespan is very condensed compared with humans, so clinical trials are more rapidly completed in dogs, and involve the same surgical, radiation, and imaging equipment (e.g., CT, MRI, PET) utilized in human oncology centers. Historically, dogs have proven to be a high value OS translational model in the development of modern limb salvage surgery methods, but also more recently in the areas of novel radiation, medical, and immunotherapeutic treatments [61–63]. As more is learnt about canine OS on a genomic level, canine models will be increasingly used in clinical trials of novel drugs proposed for targeted treatment of OS. The mutational profile of human and canine OS is similar, with mutations in *p53*, *Rb1*, *PTEN*, *MYC*, and *mTOR* found in both species [64]. More recently, the tumor suppressor Disks Large Homolog 2 (*DLG2*) was confirmed as a relevant tumor suppressor in OS using comparative genomics of humans and dogs [65••], and the histone methyltransferase *SETD2* was

identified as a potential driver of the canine disease using exome sequencing [66••]. The expression of known OS protein markers was measured by immunohistochemistry in canine OS and showed a similar profile to human OS [67]. Comparisons have also been made between human and canine OS cell lines on a proteomic level, revealing shared expression of several proteins considered important for hallmarks such as metastasis [68].

The primary tumors that develop spontaneously in dogs are primarily found in the metaphysis of long bones, an anatomical location similar to a large proportion of human OS [69, 70]. This anatomic propensity is in contrast to initial murine models, where high rates of tumor formation occurred on the jaw and snout, which we now believe was the result of both the expression pattern of the *Osx1*-Cre transgene used and species differences in tooth development between mice and humans [28, 30]. The predominant metastatic profile of canine OS is to the lungs, similar to humans. In addition, because dogs develop the disease spontaneously, they can be used to study OS in the context of an intact immune system which cannot be done in PDX models [71]. Dogs also share a common environment with humans, unlike experimental models based in mice, which may be increasingly important as we further understand environmental and microbiome effects on carcinogenesis, tumor behavior, and therapeutic response.

Immunotherapy has become a breakthrough approach in cancer treatment. The power of canine OS for therapy discovery and testing is historically evidenced by the efficacy of non-specific immunostimulant muramyl tripeptide phosphatidylethanolamine (MTP-PE), now approved in Europe as mifamurtide for treatment of human OS [72, 73]. Trials using numerous immune and biotherapy approaches are underway in pet dogs with cancer, including canine OS treatment [63]. *HER2* has been studied as a relevant target in OS, including the canine disease, and a recombinant vaccine has been developed using an attenuated *Listeria monocytogenes* vector expressing this target. This vaccine completed phase I clinical trial in 18 canine OS cases, with promising clinical results, as well as evidence of T lymphocyte infiltration, and interferon gamma-specific responses [13]. This treatment has now gone on to evaluation following chemotherapy in the adjuvant setting in a larger canine OS trial. A recombinant *Salmonella* expressing IL-2 vaccine was also tested in the OS adjuvant setting with doxorubicin chemotherapy in 19 dogs, with promising clinical results in an admittedly small study. The safety endpoints were met, permitting further evaluation of this novel therapeutic approach in larger future trials [14]. Another biotherapy currently being investigated in canine OS is the use of viruses as vectors for agent delivery, or mediators of cancer cell destruction (oncolytic virotherapy). Delivery of a replication-deficient adenoviral vector for intra-tumor activation of Fas ligand (FasL) was tested in a phase I trial in 56 dogs with OS, followed by standard adjuvant chemotherapy. Dogs treated with the Ad-FasL that had evidence of inflammation and high lymphocyte infiltration had significantly improved survival compared with historical controls. This result was particularly strong in tumors that expressed low FasL, which may be a marker of eligibility for this type of treatment [15]. Oncolytic virotherapy has been investigated using recombinant vesicular stomatitis virus (VSV), engineered to express interferon beta and the sodium-iodide symporter (NIS). Following preclinical studies in mice and purpose-bred laboratory dogs, a small cohort of 8 dogs with naturally occurring cancers, including OS, were evaluated for dose feasibility, pharmacokinetics, and biological activity. High VSV levels were detected in the blood, and viral shedding was not detectable [16]. Further development of these and other immune/virotherapy approaches are currently proceeding in veterinary oncology, the results of which may inform the successful design and implementation of similar trials in humans.

Conclusions

While our understanding of genetics and mutational spectrum of OS has never been better, the introduction of new therapies and improvements in clinical outcomes has not yet leveraged this knowledge. The development and validation of multiple complementary preclinical models of OS is a substantial step forward. The future improvements of outcomes for patients with OS, and metastatic OS in particular, are able to make use of the strengths of each of these models and the rapid advances in technologies allowing genome-wide screening, sequencing, and immunotherapy. The near real-time monitoring of the in vivo evolution and behavior of OS in human patients is also now possible [74], and concurrent developments in tumor monitoring such as circulating tumor DNA will allow a detailed understanding of the complexities of therapy response. The potential for personalized medicine has been a goal of the genomic area, and such approach has begun to be implemented in the management of canine OS. In a multi-institutional effort, genomic profiling of individual dog OS cases and development of a personalized medicine algorithm (termed Pmed) concluded that such an approach is feasible, with good quality data obtained in an average turnaround time of 5 business days [75]. Another recent study employed a gene expression model to predict sensitivity to doxorubicin and platinum chemotherapy using a retrospective comparison of clinical outcome in canine OS cases. Longer disease-free intervals were seen in cases where the bioinformatic predictions matched the drug received [76]. Equally importantly given the rare status of human OS and the limited numbers of patients eligible for clinical trials is the capacity to leverage these preclinical models for prioritization of agents for testing. A good example is the testing of the multi-targeted kinase

inhibitors sunitinib and its sister compound toceranib, which demonstrated efficacy in murine models but this was not able to be demonstrated in larger scale canine clinical trials [77•, 78•, 79•, 80•]. The full utilization of these preclinical platforms offers a means to prioritize agents for clinical trials and to improve OS outcome across multiple species.

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Compliance with Ethical Standards

Conflict of Interest Carl Walkley reports grants from National Health and Medical Research Council, Australia, Victorian Cancer Agency Research Fellowship, and Victorian State Government Operational Infrastructure Support, during the conduct of the study.

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