Published in final edited form as:
Expert Opin Drug Discov. 2013 October ; 8(10): 1181–1189. doi:10.1517/17460441.2013.817988.

Xenograft and genetically engineered mouse model systems of osteosarcoma and Ewing's sarcoma: tumor models for cancer drug discovery

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Abstract

Introduction—There are > 75 histological types of solid tumors that are classified into two major groups: bone and soft-tissue sarcomas. These diseases are more prevalent in children, and pediatric sarcomas tend to be highly aggressive and rapidly progressive. Sarcomas in adults may follow a more indolent course, but aggressive tumors are also common. Sarcomas that are metastatic at diagnosis, or recurrent following therapy, remain refractory to current treatment options with dismal overall survival rates. A major focus of clinical trials, for patients with sarcoma, is to identify novel and more effective therapeutic strategies targeted to genomic or proteomic aberrations specific to the malignant cells. Critical to the understanding of the potential for targeted therapies are models of disease that are representative of clinical disease and predictive of relevant clinical responses.

Areas covered—In this article, the authors discuss the use of mouse xenograft models and genetically engineered mice in cancer drug discovery. The authors provide a special focus on models for the two most common bone sarcomas: osteosarcoma (OS) and Ewing's sarcoma (ES).

Expert opinion—Predicting whether a new anticancer agent will have a positive therapeutic index in patients with OS and ES remains a challenge. The use of mouse sarcoma models for understanding the mechanisms involved in the response of tumors to new treatments is an important step in the process of drug discovery and the development of clinically relevant therapeutic strategies for these diseases.

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Declaration of interest
The authors are supported by Nemours Biomedical Research.

Keywords

bone sarcoma; Ewing's sarcoma; genetically engineered mouse; osteosarcoma; soft-tissue sarcoma; xenograft

1. Introduction

Sarcomas can occur in different tissues, such as bone (osteosarcoma [OS] and Ewing's sarcoma [ES]), cartilage (chondrosarcoma), fat (liposarcoma) and muscle (rhabdomyosarcoma and leiomyosarcoma) and are thought to be of common mesenchymal origin [1]. Approximately 2,000 new cases of bone sarcomas and 11,000 new cases of soft-tissue sarcomas are diagnosed in the US each year [1,2]. These neoplasms occur across a wide age spectrum, affecting children, adolescents, young adults and the elderly. Surgery and neoadjuvant chemotherapy are used in the treatment of OS, while neoadjuvant chemotherapy, surgery and radiation are used in the treatment of other subtypes such as ES and rhabdomyosarcoma. Within the past two decades, detailed histological and genetic studies have provided well-defined cytogenetic and molecular genetic information for the classification of sarcomas based on tissues and cell types from which they arise. One-third of sarcomas show specific genetic alterations and relatively simple karyotypes with translocations which produce defining gene fusions (e.g., EWS-FLI1 in ES, ASPLTFE3 in alveolar soft part sarcoma, JAZF1-JJAZ1 in endome-trial stromal sarcoma and HMGIC fusions in liposarcoma [3] or specific genetic mutations (e.g., c-kit in GIST) [4]. The remaining two-thirds of sarcomas show complex karyotypes with multiple chromosomal rearrangements, duplications and deletions (e.g., OS and leiomyosarcoma) [5].

Discovery of the protein tyrosine kinase c-kit expression in gastrointestinal stromal tumors (GISTs) has transformed treatment of this refractory and almost invariably fatal disease through targeted therapy using imatinib mesylate and other targeting agents [6]. Imatinib is also used in the management of dermatofibrosarcoma protuberans (DFSPs), in which the platelet-derived growth factor (PDGF) signal transduction pathway is activated. However, other sarcomas that are unselected for a molecular target do not yield similar responses. Notably, after relapse from first-line standard therapy, few effective salvage second-line therapies are available for most sarcomas, and no other target has been identified in GIST and DFSP. The Sarcoma Alliance for Research through Collaboration (SARC) and the Children's Oncology Group (COG) are two main organizations involved in planning clinical trials for the development of new therapeutics for adults and children with sarcoma. The availability of numerous novel and approved drugs combined with the small number of patients with these diseases that are eligible for clinical trials have contributed to the advancement of xenograft and transgenic in vivo mouse models for exploratory preclinical testing. Insights into the benefits and risks of drugs and other therapeutic strategies are provided by these studies to aid the early phase clinical testing of these agents in an efficient manner [7].

The standard *in vivo* sarcoma models are experimentally induced human sarcomas in rodents. Zebrafish also provide a novel context in which to study these diseases [8] and

spontaneous OS in dogs shows that there are many similarities in physiological and molecular biological characteristics between canine and human OS [9]. Early experiments which demonstrate that animal models subjected to high dose radiation developed sarcomas [10] identified one cause for the natural development of some sarcomas. More robust and reproducible *in vivo* models were later developed from patient-derived cell lines in immunodeficient mice to establish human xenograft tumors [11,12]. Employed to provide a better under standing of tumorigenesis, disease progression, drug response and drug resistance, the available and highly specific experimental models possess some inherent limitations related to tumor stage, genetic background and growth conditions.

The close genetic and physiological similarities between mice and humans, as well as the ease with which the mouse genome can be manipulated and analyzed, have led to the development of genetically engineered mouse (GEM) models as a key mammalian model system for genetic research. New sarcoma models permit the study of sporadic disease by specifically controlling timing and location of mutations. The ability to create transgenic mice harboring-specific sarcoma tumor fusions allows tumors to develop in their native microenvironment in animals with intact immune systems to more closely recapitulate human sarcomas. The expression profiles of lineage-specific genes of GEM models of OS show many similarities to the human disease [13].

These GEM models facilitate *in vivo* imaging of both primary and metastatic tumor development from early stages of development and are effective to guide the discovery and development of molecular-based therapies against potential targets leading to sarcoma formation. However, current preclinical and clinical studies demonstrate that individual patient-derived xenografts or GEM models, though informative, do not fully reproduce clinical behavior and may fall short of being predictive of many aspects of human response. Determining which model is best-suited for the evaluation of different therapies and targets and understanding the correlation between experimental risk and toxicity profiles and clinical efficacy are necessary. In this article, we discuss how xenograft and GEM mouse models of OS and ES currently inform the discovery of new drugs and therapies for clinical development.

2. *In vivo* mouse model systems for osteosarcoma

The most common form of bone sarcoma is OS, which accounts for approximately 35% of bone tumors diagnosed each year in the US. Numerous chromosomal imbalances such as amplification of 8q and 15q and loss of 10p and 13q, and changes in DNA copy number, have been reported in high grade OS [14]. Target genes of recurrent amplifications including mouse double minute 2 homolog (MDM2), protein-coding primase DNA polypeptide 1 (PRIM1), cyclin-dependent kinase 4 (CDK4) and sarcoma amplified sequence (SAS) [15] have also been identified in some tumors. More recent studies using FISH analysis show that cyclin-D2 (CCND2), ETS variant gene 6 (ETV6) and Kirsten rat sarcoma 2 (KRAS2) were differently amplified in low-grade and high-grade OS, [16], and overexpression of MET and FOS [17] is detected in some cases. In addition, like many bone tumors, some incidences of OS occur in the setting of inherited tumor syndromes. Studies of Li-Fraumeni syndrome, hereditary retinoblastoma and RecQ helicase disorders (Rothmund-Thomson syndrome),

along with sporadic OS, have delineated the roles of both p53 and retinoblastoma protein (Rb) in disease pathogenesis [18]. Human OS is an aggressive disease of unknown origin and complex cytogenetics and a prototype for the challenges in developing tumors in mice that recapitulate these neoplasms with complex karyotypes.

2.1 OS xenografts

Human OS cell lines or patient-derived OS tumors implanted subcutaneously in immunocompromised mice are routinely used to generate human xenograft tumors. These tumors are enriched for neoplastic cells with minimal contaminating mouse stromal and vascular tissue [19]. Validation of these *in vivo* models by gene expression profiling confirms that gene expression patterns and copy number alterations are preserved in patient-derived OS cell lines and xenograft tumors [20,21] and are therefore relevant for molecular and drug screening studies. Some concerns about this approach are the repeated passage of immortalized OS cell lines in tissue culture or successive xenotransplantations of patient-derived tumors, which may result in genotypic and phenotypic changes of the original malignant cells. Mislabeling is also reported as a well-known phenomenon. The use of well-characterized cell lines and patient tumors with low-passage greatly improves the fidelity of these xenografts when compared to the human disease [13]. More recently, xenografts panels, such as those of the pediatric preclinical testing program (PPTP), have been developed [22]. This method also provides comparative screening of multiple tumors, which further leverages the molecular diversity in OS for improved drug evaluation. This is important since individual cancers of the same type can display heterogeneous drug responses. These *in vivo* murine models allow the rapid testing of single-agent dose-responses, combinations of agents, routes of administration and surrogate endpoint biomarkers to generate initial pharmacokinetics (PKs) and toxicology data.

Traditionally, mouse OS xenografts have been used to optimize effects of standard chemotherapeutic drugs, cisplatin, doxorubicin, ifosfomide and methotrexate [23]. The evaluation of other drugs such as vincristine, vinblastine, etoposide, bleomycin, mitomycin C and actinomycin D has contributed to the identification of critical genes in some tumors that are linked to drug resistance [24], including P-glycoprotein, multidrug-resistance related protein (MRP) 1 and multi-drug-resistance (MDR) 1 gene. Resistant xenograft tumors are used as experimental model systems to explore the mechanisms of drug resistance and to study resistance-modifying agents for OS [24,25] to enhance tumor sensitivity to treatments. Further, while subcutaneous xenograft models are not typically considered highly predictive for targeted cancer therapies in humans, modification of standard OS xenografts by depletion or amplification of target genes in cell lines by RNA interference (RNAi), plasmid overexpression or mi-RNA-based techniques, which alter gene expression provide a suitable platform to facilitate the investigation of targeted therapies. The knockdown of BMI-1 (B lymphoma Mo-MLV insertion region 1 homolog) in OS cells demonstrates that BMI-1 protein sensitizes OS cells to cisplatin-induced apoptosis by inhibition of PI3K/AKT pathway [26]. These studies establish critical biomarkers which may stratify patients most likely to respond to a specific therapy while sparing those who may show little therapeutic benefit.

The most common site to which OS metastasizes is the lungs and metastatic disease evident at presentation indicates more aggressive disease and poorer prognosis. Since molecular differences occur between the primary tumor and pulmonary metastatic lesions, specific OS xenograft models are used to evaluate more effective therapeutic strategies that suppress metastasis [27]. In *in vivo* models, disseminated human cells originating from a primary patient-derived tumor are likely to represent true metastatic tumors. The 143B virally transformed HOS cell line is also used to grow metastatic xeno-grafts [28]. Orthotopic models such as the K7M2 murine OS model are favored to study spontaneous micrometastases in the absence of a primary tumor allowing the assessment of novel therapeutics in the context of minimal residual disease [29,30]. Cell lines from the primary tumor and metastatic lesions of an OS patient can provide comparative OS xeno-graft models for screening effective treatment strategies against the primary tumor and metastasis [31]. Some models are well-suited for studies incorporating immunotherapy into current treatment strategies. Biologic therapy for OS using genetically modified T-cells targeting interleukin-11 receptor α-chain [32] and immunotherapy targeting HER2 with genetically modified T-cells [33] both inhibit proliferation of OS tumors and may represent novel therapeutic approaches for OS patients with pulmonary metastasis.

The major limitations to the interpretation of data from these OS models may lie in inherent biological differences in the stroma and vascular environment between xenografts grown in ectopic sites in mice and the human tumor environment which may impact the response to therapy [34]. Other weaknesses to xenograft models are that the influence of germline genetic variation on tumor response to drug cannot be assessed in a xenograft model and cancer therapeutic agents which act through the immune system cannot be tested in immunodeficient mice [35]. Nonetheless, the potent anti-cancer activity of agents in standard, drug-resistant and metastatic in vivo mouse OS xenograft models (Table 1) supports testing the efficacy of these drugs to aid the early-phase clinical assessment in this disease.

2.2 Genetically engineered mouse OS models

A range of experimental approaches has been used to develop genetically engineered mouse models of OS [35]. These generally mimic gene deletions (deletion of TP53) [18], gene amplification (overexpression of c-Fos) [17] and point mutations (heterozygous mutation of Nf2) [36] that predispose patients to this disease. Murine GEM model systems for OS show many genetic and histological similarities to the human disease [37]. Germline and conditional gene ablations for genes which induce aneuploidy, tumor suppressors and factors that function in normal mesenchymal differentiation drive malignant transformations in various mouse models, especially in combinations that silence the p53 and Rb1 pathways. Conditional models of p53 and Rb knockout mice demonstrate the defining features of human OS, including cytogenetic complexity and comparable gene expression signatures, histology, and metastatic disease [37]. Other model systems generated from the conditional inactivation of p53 and Rb1 in osteoblasts [38] and in Sca-1 positive mesenchymal stem/progenitor cells [39] provide appropriate genetic models of inherited disease (Table 1). In addition, chemical carcinogens, external beam radiation and bone-seeking heavy metal radioisotopes also induce OS in wild-type mice [40,41].

While these GEM models of OS recapitulate multiple defining features of human OS, pathway differences between the human and mouse species, the observation of spontaneous OS in rodents and lack of true lamellar bone in the mouse are some notable weaknesses of these models. In one model, tumors were located in the jaw and head [38] in contrast to the ends of the long bones in human OS, and metastasis was primarily to the liver. Current GEM models are generally considered to be the most genetically and histologically similar to human OS and provide opportunities to test or screen for novel therapeutic agents to treat this disease.

3. *In vivo* mouse model systems for Ewing's sarcoma

3.1 Ewing's sarcoma

ES is the second most common bone malignancy in children and young adults after OS and accounts for ~ 16% of bone sarcomas in children. ES has epidemiological features similar to OS, but it tends to arise in the diaphysis [42]. Treatment consists of chemotherapy combinations of vincristine, doxorubicin and cyclophosphamide, which are altered with combinations of ifosfamide and etoposide [43]. The predicted progenitor cells of this neoplasm are human mesenchyme stem cells (MSC). ES was the first sarcoma to be defined by a specific translocation [44]. The cytogenetic abnormality of this disease is related to the presence of a balanced t(11;22) chromosomal translocation expressing the EWS-FLI1 chime-ric fusion protein [45]. A reciprocal translocation mechanism that results in an EWS-ERG fusion gene has also been described [46], and FEV, ETV1 and ETV4 fusions have been reported [47]. EWS-FLI1 is a transcription factor oncoprotein that binds RNA helicase A and activates numerous genes involved in proliferation, cell differentiation, and cell survival such as IGF-1, NKX2, TOPK, SOX2, and EZH2 and represses genes involved in apoptosis and cell cycle arrest including IGFBP3, p57kip, p21, and TGFB2 [48]. Related pathways involved in disease pathogenesis include p53, INK4A, IGF-1/IGF-1R, bFGF, CD99, other tyrosine kinase and Wnt pathways [49].

3.2 ES xenograft models

In contrast to OS which has no pathopneumonic mutation, ES tumors express the EWS-FLI1 fusion protein product of the reciprocal translocation of the *EWS* gene on chromosome 22 and the *FLI1* gene on chromosome 11. ES cell lines and patient-derived primary ES tumors or lung metastases harboring the EWS-FLI1 fusion are used to grow *in vivo* ES xeno-graft tumors. The EWS-FLI1 protein can transform primary bone marrow-derived mesenchymal cells to form ES-like tumors in mice [50]. However, the induction of EWS-FLI1 alone does not transform human MSC [50] indicating that the development of ES involves multiple prooncogenic events. The oncogenic fusion proteins drive transcription of genes that contribute to the malignant phenotype and confer resistance to DNA damaging agents, suggesting that modulation of proteins could be a possible therapeutic strategy.

The search for therapies targeting EWS-FLI1 or EWS-FLI1 mediating signaling is a promising strategy. EWS-FLI1 transcription factor is considered a potentially ideal therapeutic target, and the development of effective drugs is being investigated. Other strategies based on antisense cDNA and siRNA oligonucleotides against EWS-FLI1 have

been used to modulate this target in ES xenografts. While these agents reduce EWS-FLI1 expression and increase survival rates in mice with ES [51], delivery systems to the tumor cells which express this gene are being tested using nanoparticles and nanocapsules as nonviral delivery systems [52]. More recently, the YK-4-279 compound which blocks binding of RNA helicase A and EWS-FLI1 reduced growth in ES xenograft tumor models [53]. This is the first EWS-FLI1 targeted therapy with the potential for advancement for clinical studies.

Studies using agents targeting candidates involved in molecular events leading to disease progression have shown clear pre-clinical and clinical responses. Antitumor effects of inhibitors of the tyrosine kinase receptors, such as IGF-1R, c-kit, PDGFR, VEGFR, or the mTOR signaling pathway, proteasome, angio-genesis and stress response proteins have all been studied in ES xenografts. IGF-1R targeting antibodies have undergone single-agent and combination testing in ES xenograft models [54-57]. High throughput proteomic techniques have identified new therapeutic targets in ES and novel targets such as HSP90 have been shown to be of relevance to ES [58-60].

3.3 Genetically engineered mouse ES models

To date, no GEM models have been successfully developed for ES. Conditional expression of the EWS-FLI1 gene resulted in bone abnormalities but caused embryo lethality [61]. In another approach, Cre-mediated activation of EWS-FLI1 resulted in rapid development of myeloid/erythroid leukemia in mice [62] suggesting that activation of EWS-FLI1 has a role in this disease. Since early precursor cells are believed to be MSCs, attempts were made using EWS-FLI1 expressed exclusively in MSCs [63]. Failure of tumors to develop in mouse hosts following infection of stem cells with a retrovirus containing EWS-FLI1 suggests ES tumor growth only occurs in the presence of EWS-FLI1 when specific mutations already exist in a given cell [50]. Cooperative interactions between EWS-FLI1 and p53 deletions are also being studied as a potential model [61].

4. Conclusion

These studies demonstrate that well-characterized cell lines and patient-derived xenograft mouse models of OS and ES depict many aspects of the histology, molecular and pharmacology profiles of these neoplasms. The mouse model systems described here provide an experimental framework to support the preclinical testing of drugs. GEM models of OS further allow assessment of the tumor microenvironment and immune system for therapeutic immune-modulatory activities and evaluation of antitumor activity related to drug-dependent cell cytotoxicity. Delineating the predictive potential of individual mouse models and integrating the responses of relevant disease models are critical to the overall assessment of drug efficacy (summarized in Table 2). The ongoing refinement of transplantable tumor mouse models and transgenic and knockout mice to recapitulate various genetic alterations specific to these cancer types will further optimize experimental models to aid drug discovery and development.

5. Expert opinion

Many cancer therapeutics that are effective in adult clinical trials have not demonstrated similar successes in pediatric patients with OS and ES. This supports the rationale for independent testing of novel preclinical treatments for these diseases in suitable *in vivo* model systems, for a better understanding of anticancer activity. Advances in the development of experimental OS and ES mouse models have progressed rapidly from transplantation of malignant human tumors in immunocompromised mice in the 1960s to the more sophisticated genetically engineered mice in the 1980s. While the natural development of these diseases is still unknown, these advances allow better modeling of tumor development that reflect many histopathological features of the human diseases. Further, it is widely believed that the application of novel cancer therapeutics can be successfully explored in predictive *in vivo* mouse models for OS and ES, leading to the identification of better therapeutic and diagnostic strategies.

One criterion in determining the efficacy of novel agents in clinical trials for solid tumors is the evaluation of tumor response for patients with measurable disease. This is also a key measurement in preclinical studies. Tumors grown to a predetermined size in murine OS and ES model systems are treated with agents at specific doses and schedules and the tumor growth is assessed. Xenograft models such as those of the PPTP have established PK and pharmacodynamic (PD) endpoints with predictive drug efficacies in patients. Agents that demonstrate a high level of activity against tumors may elicit significant toxicity in patients, while insufficient activity by other agents may also suggest limited clinical benefits. The main goal is to identify effective drug concentrations, which may indicate optimal activity of these agents with the potential to match therapeutic approaches. In addition, these experiments could identify critical pharmacological biomarkers to assist in the optimization of drug concentrations.

Many standard and novel agents which have undergone preclinical evaluation have shown promise in clinical investigations for these diseases. These include dasatinib (Src inhibitor) [64,65], SCH717454 (IGF-1R receptor inhibitor) [54], and rapamycin (mTOR inhibitor) [66,67]. Several rapamycin analogs including temsirolimus (CCI-779) [68], everolimus (RAD-001) [69] and deforolimus (AP23573) [70] tested in planned clinical trials, demonstrate that these agents are also well-tolerated, with antitumor activity in adult and pediatric patients. In contrast, early preclinical testing of the multi-kinase inhibitor sorafenib supported testing of this drug as a potential therapeutic option in metastatic or relapsed OS patients unresponsive to standard treatments [71]. In patients, disease stabilization and tumor shrinkage were short-lived and drug resistance was observed [72]. The recent evaluation of sorafenib in OS xenografts in combination with everolimus showed enhanced antitumor and antiangiogenic effects, and reduced metastatic colony formation by complete inhibition of the mTOR pathway [73]. These and other early phase clinical reports highlight opportunities for the development of combination therapies utilizing these agents and conventional chemotherapeutic regimens or targeted therapies in experimental testing.

One prevailing concern that could affect the ability to translate good preclinical responses in mice to the clinical setting is interpretation of preclinical data. While validated OS and ES

murine models are proven robust and reproducible platforms for drug testing, they represent one component of an experimental study. Experimental designs (which murine model is best suited and when therapeutic regimens are started), data analysis (statistics) and data conclusions may result in false-positive and false-negative preclinical reports. Further, a single OS and ES mouse model may not be the best experimental model for these neoplasms which are characterized by complex genotypes and phenotypes. Significantly, Hingorani *et al*. [27] showed effective target inhibition in primary tumors by the Src inhibitor dasatinib, with no effect on pulmonary metastasis. Differences in gene expression patterns between the primary tumor and metastasis highlight the importance of the selection of appropriate *in vivo* model systems for the study of metastasis. The genetic composition of an individual xenograft tumor influences drug response and ideally, diverse tumors for comparative drug screening (as evident in the PPTP panels) provide more comparative and reliable testing. Similarly, the use of several strains of mice with different genetic backgrounds is expected to better model a human patient population. Advanced imaging modalities (optical, ultrasound, PET, CT and MRI) to monitor tumor growth and drug stability *in vivo* will also augment data interpretation.

The ability to predict some of the adverse drug effects in the clinical setting may also improve the quality of preclinical data to better understand benefits for patients. The application of meaningful experimental endpoints will strengthen the use of *in vivo* models for preclinical drug development for these sarcomas. Drug evaluation in other model systems can be conducted for a more comprehensive understanding of drug response. Severe inflammatory stress produces genetic imbalances which affect critical cellular functions and pathways [74], which can complicate the already complex gene expression patterns in OS and ES. The use of joint inflammation models, neuropathic pain models and spontaneous pain models could inform preclinical studies about these parameters in drug development [75]. As with any experimental animal system, murine models of acute, tonic and chronic pain also present limitations (sex, genetics, testing environment and social modulation). More recent animal models (e.g., operant methods and spontaneous behaviors) provide sensitive and rapid evaluation of new therapeutic agents *in vivo* [75].

In summary, the testing of new therapies in a single *in vivo* mouse model system of OS and ES is not expected to fully predict clinical behavior and outcome for patients. Each animal model offers opportunities and limitations and relevant conclusions can only be made in the context of an individual study. Not all the factors discussed in this review pertaining to mechanisms of response and resistance can be addressed in a single experimental design. Understandably also, questions of short and long-term side-effects will only be answered in clinical trials. Thus, combining of resources through collaborations, crosstalk and correlation of preclinical and clinical data is necessary to improve the reliability and predictability of preclinical studies to minimize risks and maximize benefits to patients. We suggest that combination therapies and drug screening in multiple predictive preclinical models may be the best systematic approach to improve the overall success rate for novel drug candidates for these diseases.

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Article highlights

- Many *in vivo* mouse OS and ES xenograft models and OS GEM models are considered appropriate for preclinical testing of novel drugs and therapies for early phase clinical assessment for these diseases.
- The development of multiagent therapies utilizing new drugs and conventional chemotherapeutic regimens or targeted therapies is expected to be the most effective therapeutic strategy in OS and ES.
- The use of several strains of mice with different genetic backgrounds is expected to better model a human patient population.
- Preclinical studies of mechanisms of response and resistance would aid in framing decisions when translating these treatment strategies to the clinic.
- The true establishment of drug efficacy and safety is achieved in clinical trials.

This box summarizes key points contained in the article.

Table 1

Summary of xenograft and GEM mouse models of OS and ES discussed in review.

Malignancy	Mouse model	Significance to preclinical studies of drug development	Mouse strain or genetic alteration	Cell line	Refs.
OS	Subcutaneous	Comparative screening of multiple tumors of different karyotypes	CB17SC-M scid$^{-/-}$	Patient-derived (OS-1, OS-2, OS-17, OS-9, OS-33, OS-31, OS-29)	[22]
	Orthotopic	Evaluation of spontaneous metastasis	C.B-17	143B	[27]
			BALB/c	K7M2	[30]
			C3H	DLM8	[29]
			NOD/SCID	143B	[28]
		Modification of targets by gene silencing or overexpression	Unknown	SaOS-2	[26]
	Metastatic	Test metastatic xenografts generated from metastatic cell lines	BALB/c	Zos-M	[31]
			Nu/nu	KRIB	[32]
			NOD/SCID	143B	[33]
	Resistant	Study of mechanisms of resistance and resistance -modifying agents	C3H/Sed	MOS/ADR1	[25]
			C3H/Sed	MOS/ADR2	
	Spontaneous	Evaluation of naturally developing tumors	Fischer		[40]
	GEM	Study tumors in native environment and with intact immune system	Osx-Cre+p53fl/flpRbfl/fl		[38]
			Osx-Rbc/c;p53c/c		[39]
ES	Subcutaneous	Screening of tumors expressing EWS-FLI1	BALB/cJHanHsd-SCID	Mouse MPC	[50]
		Modification of EWS-FLI1 expression by gene silencing	BALB/c nu/nu	SK-N-MC	[51]
		Comparative screening of multiple tumors	CB17SC-M scid$^{-/-}$	Patient-derived (EW-5, EW-8), SK-NEP-1, TC-71	[22]
	GEM	Study tumors in native environment and with intact immune system	Prx1-Cre EWS-FLI1		[61]

MPC: Mesenchymal progenitor cell.

Table 2

Preclinical findings and interpretation of data.

Preclinical data	Interpretation
Tumor inhibition and regression	Drug efficacy
Pharmacokinetics	Tolerability or toxicity profiles
Pharmacodynamics	Target expression relevant for drug action
Tumor growth	Resistance—Target addiction; activation of survival pathways
Biomarkers	Predictive of response to drug