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Identifying novel therapeutic agents using xenograft models of pediatric cancer

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Abstract

In the USA, the overall cure rate for all childhood cancers is seventy percent, and in many patients that ultimately fail curative therapy, initial responses to current multimodality treatments (surgery, radiation therapy and chemotherapy) is good, with overall 5-year event-free survival approaching 80 %. However, current approaches to curative therapy result in significant morbidity and long-term sequelae, including cardiac dysfunction and cognitive impairment. Furthermore, dose-intensive chemotherapy with conventional agents has not significantly improved outcomes for patients that present with advanced or meta-static disease. Classical cytotoxic agents remain the backbone for curative therapy of both hematologic and solid tumors of childhood. While 'molecularly' targeted agents have shown some clinical activity, responses are often modest and of short duration; hence, there is a need to identify new classes of cytotoxic agent that are effective in patients at relapse and that have reduced or different toxicity profiles to normal tissues. Here we review the pediatric pre-clinical testing program experience of testing novel agents, and the value and limitations of preclinical xenograft models and genetically engineered mouse models for developing novel agents for treatment of childhood cancer.

Keywords

Human tumor xenografts; Cytotoxic drugs; Pediatric tumor xenografts

Introduction

Developing new therapeutics for treatment of childhood cancer presents challenges unique to these rare diseases. In the USA, approximately 12,500 new cases of cancer are diagnosed annually in patients 21 years or younger. Since the mid-1960s, cytotoxic drug/radiation therapy has dramatically increased survival, with overall survival being 70 % and 5-year event-free survival approaching 80 %. For acute lymphoblastic leukemia, the 5-year event-free survival (EFS) is 85–90 %, whereas one-half to two-thirds of children with Ewing sarcoma, rhabdomyosarcoma or osteosarcoma are surviving disease-free for prolonged

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Compliance with ethical standards

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periods after aggressive treatment with surgery, radiation and multiagent chemotherapy. While dose intensification/compression and introduction of new agents continues to decrease cancer mortality in children [1], the limits of cytotoxic therapy may be close to maximal. However, these therapeutic modalities are associated with significant mortality and often long-term debilitating sequelae [2]. While the overall increase in survival has been a medical success, children with advanced or metastatic disease at diagnosis remain at risk with a poor prognosis, and relatively little improvement in outcome has been leveraged for this group of patients.

As with advanced solid tumors, childhood brain tumors still present a challenge. Current therapies, where curative, may leave children with significant long-term health issues including neurocognitive dysfunction, endocrine deficits, visual deficits, vasculopathy and secondary cancers [3, 4]. For most childhood tumors therapy is surgery followed by radiation and chemotherapy, dependent upon whether total gross resection is possible. The drugs used most frequently remain classical cytotoxic agents, cyclophosphamide, etoposide, cisplatin, vincristine, and for brain tumors temozolomide and lomustine. While these agents are clearly a component of curative therapy, they also lead to long-term adverse effects, infertility, secondary cancers and, potentially, with radiation, endocrine and neurocognitive degeneration in survivors of brain tumors. Further, estimates indicate that the incidence of radiation necrosis in normal brain ranges from 3 to 24 % of patients following focal irradiation [5–7] and may be up to threefold higher with concurrent chemotherapy [7].

Failure of curative therapy is largely a consequence of drug resistance. Whether this results from selection of a preexisting clone, or through therapy-induced mutation, remain to be extensively characterized. The second problem is the limited repertoire of active antineoplastic agents, targeted for childhood cancers, making it difficult to develop effective therapy for resistant tumor subtypes. As in the management of adult cancers, the development of novel therapies for childhood solid tumors will require a more complete understanding of the biologic characteristics that confer the malignant phenotype that can be used to guide the integration of cytotoxic and molecularly targeted therapies most likely to confer clinical benefit.

An additional restriction on drug development is that cancers of childhood respond well to drugs of established efficacy, resulting in cure of a substantial number of patients. This ethically precludes the use of ‘experimental’ agents at diagnosis and severely restricts the number of clinical trials that can be undertaken to test new therapeutics. Thus, the pediatric preclinical testing program (PPTP) was established to see whether preclinical models (exclusively childhood tumor xenografts) could assist in identifying novel agents that could be moved rapidly into pediatric cancer clinical trials. Here we review the PPTP experience in identifying novel ‘cytotoxic’ agents that have particular efficacy against childhood cancer models.

PPTP tumor models

Over 150 xenograft models have been developed by the members of the PPTP consortium, and characteristics of models used in screening can be found at: <http://gccri.uth-scsa.edu/pptp/img/docs/demographics.pdf>. Most are transplants from patients directly into mice

(patient-derived xenografts, PDX), and there is an equal distribution of models derived at diagnosis and relapse. A full screen utilizes 49 models in mice and 23 cell lines. <http://gccri.uthscsa.edu/pptp/img/docs/demographics.pdf>.

Considerations in establishing a screen

When we established the in vivo screen, there was a general consensus that preclinical animal models had failed to accurately predict clinical efficacy. We therefore considered problems associated with prior screening approaches and what factors were contributing to their failure to accurately predict for active agents in human clinical trials. In terms of importance, we considered that: (1) the models had not encompassed the molecular/genetic characteristics of the human cancer type; (2) drug exposure in the preclinical models may significantly exceed that achieved in humans (i.e., species differences in pharmacology or tolerance); and (3) criteria for defining ‘active’ in preclinical models were less stringent than clinical criteria for defining ‘activity.’ While there were other factors, we will address some of the pitfalls if these considerations are not factored into screening.

Identification of ‘classic’ cytotoxic agents

As part of any evaluation of models to be used for identification of ‘effective’ therapeutics, it is imperative to demonstrate that known clinically ‘active’ drugs can be identified by the screen. For childhood solid tumors, most treatment protocols use cyclophosphamide, vincristine, cisplatin, as well as topoisomerase II inhibitors (etoposide, doxorubicin) or topoisomerase I inhibitors (topotecan, irinotecan). In PPTP, and other studies [8–10], these agents are identified as being active in one or more panels of tumor models. This builds confidence that the models recapitulate the known clinical drug sensitivities. Further, synergistic activity between vincristine and camptothecin agents (topotecan, irinotecan) identified in xenograft models [11] is now routinely used for treatment of childhood cancers. Notable for their absence from the list of agents tested are doxorubicin and etoposide. Doxorubicin is highly toxic to severe combined immunodeficient (SCID $-/-$) mice; hence, exposure in mice is lower than in humans, whereas etoposide is cleared rapidly from mouse plasma and exposure in mice is not relevant to human exposure [12, 13]. Temozolomide at dose levels that exceed systemic exposures achievable in humans also shows significant and broad-spectrum activity against solid tumor and ALL models. However, at doses more relevant to human exposure, only models with known deficiency in the DNA repair suicide-protein MGMT are sensitive [14]. Thus, the preclinical screen identifies drugs that are active clinically, but it can also overpredict (false positive) or under-predict (false negative) based upon drug tolerance of the mouse. It is thus imperative that relative systemic exposures causing tumor regressions in mice are related to systemic exposure in human when pharmacokinetic data are available [15]. Obtaining such pharmacokinetic data at a relatively early stage in clinical drug development may identify those agents that will ultimately fail in the clinic for lack of efficacy. While such data are not available before testing in man, such ‘retrotranslation’ can be valuable in making informed decisions about advancing an agent to adult phase II testing, or initiating pediatric trials [16, 17].

Identification of novel cytotoxic agents

Classical cytotoxic agents used in therapy of childhood cancer have in general modified cellular processes such as DNA metabolism/replication or mitosis. DNA may be modified through direct binding (cyclophosphamide, cisplatin), incorporation into DNA (cytosine arabinoside) or modification of DNA metabolizing enzymes such as topoisomerases (topotecan, etoposide), leading to induction of repair processes, and activation of TP53-dependent and TP53-independent pathways leading to cell death or survival. While these agents tend to damage normal tissues having high rates of cellular proliferation (bone marrow, intestine), the consensus was that ‘selectivity’ for tumor was based upon rapid kinetics of tumor cell proliferation. However, it is now becoming clear that tumor responsiveness may be a consequence of defects in DNA damage repair processes that lead to hypersensitivity (e.g., BRCA1/2 mutations). Similarly, the selectivity of antimitotic agents was considered to relate to rates of tumor cell proliferation. However, selectivity may be a consequence of tumor cells being deficient in drug transporters that efflux drugs, whereas normal tissues are protected by these efflux pumps. With an increasing understanding of the pathways leading to cell death (apoptosis, necrosis, etc.), the classical definition of a ‘cytotoxic’ drug needs to be expanded to include direct inducers of apoptosis or other death pathways. These include inhibitors of anti-apoptotic proteins (e.g., ABT-263), SMAC mimetics (e.g., TL32711), MDM2:TP53 inhibitors (e.g., RG7112, MK-8242) or small molecule inhibitors of motor proteins such as kinesins or kinase inhibitors that target essential mitotic kinases that should be classified as cytotoxic agents as they do induce cytotoxicity.

Antimitotic agents

Vincristine is the most frequently used antimitotic drug for curative treatment of both solid tumors and ALL of childhood. In contrast to vincristine, which causes microtubule destabilization, taxanes induce microtubule stabilization and have not shown significant activity against pediatric solid tumors [18–20]. Consequently, for a new antimitotic to be of interest its antitumor activity should be equal or superior to vincristine in the screen at dose levels in mice giving relevant clinical exposures. The antitumor activity of vincristine and several other tubulin binders against sarcoma models are shown in Fig. 1. In limited testing, cabazitaxel demonstrated significant activity against several solid tumor models. However, the AUC determined per cycle (Q4D ×3) would result in an exposure approximately 24-fold greater than achieved clinically when cabazitaxel was administered at 25 mg/m² to patients on an every 3-week schedule [21]. As most cytotoxic drugs have steep dose–response relationships, it is predicted that cabazitaxel will not have significant clinical utility against childhood solid tumors. In contrast, Abraxane (nab-paclitaxel) showed interesting antitumor activity. While acknowledging the multiple factors complicating comparisons of mouse to human exposure for Abraxane (for example, drug retention in tumor may be more relevant than plasma exposure), the mouse exposures per cycle appear to be somewhat greater than exposures achieved in humans by about 20 % [22]. Thus, it is reasonable to anticipate that Abraxane may have activity against childhood sarcomas. In screening for cytotoxic agents with known mechanisms of action, one is hoping to identify agents with a novel spectrum of antitumor activity that exceeds the ‘gold standard.’ For example, the tubulin binder eribulin, an FDA approved drug for third-line treatment of breast cancer, is probably the most active

agent tested in the PPTP screen at doses in mice giving relevant human drug exposures [23]. Importantly, eribulin is active against 4 of 5 Ewing sarcoma models where vincristine has no significant antitumor activity (Fig. 1). As mentioned above, xenograft models may overpredict for clinical activity as the proliferative fraction within model tumors may be greater than in the clinical situation. However, it is clear that all tubulin binders do not have significant biologic activity, as demonstrated by the lack of antitumor activity of BAL101553 in these models [24].

DNA reactive agents

PR104 is a phosphate ester of the nitrogen mustard prodrug PR104A, designed to be activated selectively under hypoxic conditions [25, 26]. When tested against panels of solid tumors and leukemias, this agent showed broad-spectrum activity [27]. From experience, this level of activity is often associated with the mouse being highly tolerant of the drug relative to human or human tumor. Indeed, the drug exposure in mice was fourfold to fivefold greater than clinical exposure. Dose response studies showed that at drug doses in mice giving relevant clinical drug exposure, only a subset of ALL models were responsive. These T cell leukemia models had high levels of an aldo-keto reductase (AKR1C3), and antileukemic activity of PR104 was highly correlated with AKR1C3 activity, suggesting that this agent may have utility in T cell ALL [28].

As mentioned above, sensitivity of tumor cells to DNA damaging agents may be a consequence of deficiencies in DNA repair pathways, for example cisplatin sensitivity in homologous recombination-defective cells due to BRCA1–2 mutations. Recently, it was found that in vitro Ewing sarcoma cells were hypersensitive to inhibitors of the DNA repair enzyme poly-ADP ribose polymerase-1 (PARP1), although the mechanism for this is unclear. Of note, the potent PARP1 inhibitor talazoparib (BMN673) had single-agent activity only against two cisplatin sensitive xenograft models in the PPTP screen [29]. One of these has a PALB2 mutation that compromises homologous recombination. Talazoparib was not active against any of the Ewing sarcoma xenograft models tested. Importantly, another PARP inhibitor, olaparib, failed to demonstrate single-agent activity in a phase II trial against Ewing sarcoma [30] suggesting that the preclinical xenograft model results are quite predictive of clinical activity. However, when talazoparib is combined with temozolomide, dramatic potentiation was found in 5 of 10 Ewing sarcoma xenograft models (Fig. 2) but not in other solid tumor or ALL models [31]. The results suggest that in a subset of Ewing sarcomas there are limited options to repair DNA damage and one such pathway is through PARP. Understanding what DNA damage repair processes are deficient in Ewing sarcoma lines responsive to this combination may allow identification of a patient population likely to benefit from this therapy. Of note, in the mouse combination of temozolomide with talazoparib necessitated an eightfold to tenfold reduction in temozolomide dose. Early-stage data from the phase I clinical trial (NCT02116777) suggest that a similar dose reduction for temozolomide may be necessary. Clearly, further studies will be necessary to identify biomarkers that distinguish tumors where the combination is highly effective from those where there is little or no response to this combination.

Novel cytotoxic agents

Classical cytotoxic agents have directly targeted DNA, DNA replication processes and the mitotic apparatus. Antimitotic agents have predominantly targeted interactions with tubulin and microtubule function. However, most molecularly targeted drugs, such as kinase inhibitors, tend to be cytostatic rather than cytotoxic unless they target driver mutations that result in cell death. Retrospective analysis of 21 signaling inhibitors, both small molecule tyrosine kinase inhibitors and antibodies that blocked ligand–receptor interactions, tested by the PPTP showed ~2 % objective responses when these agents were tested against up to 50 xenograft models. For pediatric cancer, the objective is to cure the patient; hence, targeted agents should exert cytotoxic activity. Two exceptions were the aurora kinase A inhibitor alisertib (MLN8237) [32] and the polo-like kinase-1 (PLK-1) inhibitor volasertib (BI6727) [33], both of which act on the mitotic cycle and caused complete tumor regressions in multiple xenograft models. However, for both drugs, exposures in mice significantly exceeded human exposures [33, 34], and both agents are myelotoxic in patients. A different approach to inducing tumor regression is to engage the apoptotic machinery. Here we consider three approaches, stabilization of the TP53 tumor suppressor through preventing MDM2 interaction, by trapping TP53 in the nucleus using an inhibitor of CRM1/XPO1, and inducing apoptosis using small molecule mimics of SMAC.

MDM2 inhibitors—As mutations of the TP53 tumor suppressor gene are less prevalent in pediatric compared with adult cancers [35–43], it suggests that a larger proportion of pediatric patients may benefit from pharmacological upregulation of wild-type TP53 that could initiate an apoptotic cascade. TP53 mutations are reported to occur at a higher frequency in relapsed patients [43–46], and where present have been associated with aggressive and chemo-refractory disease [43, 46, 47]. These tumors would not be sensitive to this therapeutic strategy. Thus, for most pediatric cancers reconstitution of a functional TP53 pathway is an attractive anticancer strategy. Interactions between TP53 and its two principal regulatory molecules (MDM2/MDM4) involve large protein–protein interfaces traditionally regarded as a difficult target for pharmacological intervention [48]. However, several classes of chemicals with diverse structures have been identified that are able to effectively inhibit the MDM2-mediated degradation of TP53 or inhibition of *MDM2* transcription [49]. Of these, Nutlins have demonstrated impressive activity in vivo with limited toxicity in rodent models [49], whereas most of these compounds exhibit in vitro activity. In the PPTP screen, in vitro sensitivity to the MDM2 inhibitors RG7112 and MK-8242 correlated well with wild-type TP53 status, with TP53 mutant cell lines being 10- to 40-fold less sensitive [50]. In TP53 wild-type lines, the predominant cellular response was apoptosis, consistent with the notion that elevation of TP53 would direct an apoptotic response. However, in vivo these agents induced regressions in 5 (RG7112) or 6 (MK-8242) of 26 solid tumor models, whereas both agents were highly active against ALL xenograft models, particularly those derived from infant mixed lineage leukemias [50, 51]. Of note, clinically MDM2 inhibitors induce prolonged thrombocytopenia not seen in the mouse, again highlighting a deficiency in the preclinical models. Dose-limiting toxicity was neutropenia and or thrombocytopenia in sarcoma patients and thrombocytopenia in combination with cytarabine in AML patients [52]. This appears to be an on-target toxicity as RG7112 has been shown to promote apoptosis of megakaryocyte progenitor cells and also

affected mature megakaryocytes by blocking DNA synthesis during endomitosis impairing platelet production [53]. This illustrates an interesting issue in development of targeted agents, where potency is optimized against the human target, and may be significantly less potent against the murine homolog. For example, the MDM2 inhibitor AMG-232 has approximately 40-fold less biochemical potency on murine MDM2 compared with human [54]. Thus, potentially these agents may show a far greater therapeutic efficacy in xenograft models than in clinical trials.

Selective inhibitors of nuclear export (SINE)—Selinexor (KPT-330) is a prototypical SINE that forms a slowly reversible covalent bond with XPO1 (exportin 1, CRM1) and inhibits its function. XPO1 is a member of the karyopherin- β family of proteins and plays a central role in nuclear export through forming complexes with Ran-GTP and with cargo proteins containing a leucine-rich nuclear export sequence (NES) [55, 56]. Selinexor inhibits the export activities of XPO1 by forming a covalent adduct with cysteine-528 of XPO1 [57–59] that functionally inactivates XPO1 and targets it for proteasomal degradation [58]. Mutation of cysteine-528 confers high-level resistance to selinexor [60], confirming specificity for XPO1 as the mechanism of action. Selinexor induced significant differences in event-free survival (EFS) distribution in 29 of 38 (76 %) of the solid tumor xenografts and in 5 of 8 (63 %) of the ALL xenografts. However, objective responses (partial or complete responses, PR/CR) were observed for only 4 of 38 solid tumor xenografts and 2 of 8 ALL xenografts. As shown in Fig. 3, selinexor induced sustained regressions in 3 of 4 non-glioblastoma brain tumor models and prolonged stasis in the BT-36 ependymoma model. As selinexor has good CNS penetration (CSF/plasma ~ 0.6), secondary testing in intracranial orthotopic models would appear to be warranted based on the good responses of these brain tumors in the heterotopic site (subcutaneous). Pharmacodynamic studies in sarcoma models showed that antitumor response is associated with increased p21 and cleavage of PARP. Selinexor treatment also induced nuclear localization of FOXO1, TP53, NF κ B, p-ERK1/2 and dephosphorylation of nuclear RB. These changes were associated with increased TUNEL positive cells and decreased Ki67 [61], and thus, measurement of these biomarkers in intracranial brain tumor models may serve as useful markers of drug effect.

SMAC mimetics

SMAC, the second mitochondria-derived activator of caspases, has a unique function in regulating apoptosis. In non-stressed cells, SMAC is sequestered in mitochondria and is released into the cytosol only upon induction of mitochondrial dysfunction or apoptosis [62, 63]. Cytosolic SMAC selectively binds to IAPs through conserved baculovirus IAP repeat (BIR) domains [64, 65], promoting cell death. Consequently, small molecule drugs that mimic the interaction of SMAC with IAPs (SMAC mimetics) have been designed. SMAC mimetics bind with high affinity to IAPs, including XIAP, cIAP1 and cIAP2. In some cell systems, cell death induced by SMAC mimetics requires TNF- α signaling and caspase-8 and to be independent of caspase-9 [66]. SMAC mimetics induce rapid auto-ubiquitylation and proteasomal degradation of cIAP1 and cIAP2 resulting in the activation of non-canonical NF- κ B signaling and subsequent increased TNF α production and autocrine stimulation of TNFR1 [66, 67]. This increased TNF α signaling leads to caspase-8 activation and apoptosis

as a result of the enhanced RIPK1 levels that are a downstream effect of reduced cIAP ubiquitylation of RIPK1 [67, 68].

Two SMAC mimetics, LCL161 [69] and TL32711 (birinapant, unpublished data), have been evaluated in the PPTP models. In vivo LCL161 induced significant differences in EFS distribution in approximately one-third of solid tumor xenografts (osteosarcoma and glioblastoma), but not in ALL xenografts. No objective tumor responses were observed; hence, this agent demonstrated limited single-agent activity against the pediatric preclinical models studied. TL32711 (birinapant) did not induce significant differences in EFS distribution compared to control in the evaluable solid tumor xenografts, but did retard progression in 2 of 2 of the evaluable ALL xenografts. Although of limited single-agent activity, birinapant may potentiate the activity of other agents used in the treatment of ALL. The mechanism(s) for intrinsic resistance in solid tumors remains unknown; however, methylation silencing of the caspase-8 gene has been reported in MYCN amplified neuroblastoma [70] with high frequencies in other pediatric tumors, including rhabdomyosarcoma, medulloblastoma and retinoblastoma [71]. However, methylation of the caspase-8 promoter was low in Wilms tumor, and osteosarcoma suggesting that intrinsic resistance to TL32711 or birinapant induced apoptosis in these pediatric cancer models is through mechanism(s) other than SMAC expression or caspase-8 silencing.

Orthotopic models

The advantage of orthotopic implantation (implantation to the organ site from which the tumor was removed) over heterotopic (subcutaneous) implantation is that tumors may recapitulate many tumor and microenvironment interactions. Orthotopic models are often metastatic [72–75], whereas heterotopic models more rarely metastasize [76]. The disadvantage is that orthotopic models are more labor intensive [77], and monitoring may require transfection of a marker such as luciferase, or use of ‘survival’ as an end point. If there is significant variation in the rate of tumor growth in different mice, this will necessitate larger group sizes to evaluate therapeutic effects. However, orthotopic models may be essential for validating results in heterotopic xenografts, particularly in the case of brain tumors where the blood–brain barrier may restrict drug access. However, whether models developed by intracranial injection of tumor cells or tissue fragments completely recapitulate the blood–brain barrier is unclear. For those studies, transgenic models that arise naturally in the brain, such as the *Pct/p53* medulloblastoma, may be more relevant. Of interest, the $\alpha\beta3/\alpha\beta5$ antagonist cilengitide (EMD 121974) was active against intracranial brain tumor models, whereas it was inactive in the subcutaneous site [78] supporting the notion that the intracranial site had an angiogenic microenvironment where the integrin antagonist was active. While this may be correct, it is clear that subcutaneous tumors grew more rapidly (fivefold to sixfold) than those formed in the brain after inoculation of the same number of cells in control animals. Cilengitide was not active against refractory or relapsed high-grade gliomas in a subsequent COG trial [79].

While the concept of orthotopic models is appealing, the value of these in the context of drug screening, or therapy development, remains to be validated. For rhabdomyosarcoma, a malignancy considered to be of skeletal muscle lineage, there are many reports where tumor

is engrafted into the leg muscle (for example [80]). However, rhabdomyosarcoma arises, predominantly, in areas of the body that are not necessarily associated with skeletal muscle. One of the models used was derived from a pelvic mass, whereas the other was from a bone marrow aspirate. Hence, engraftment into the skeletal muscle site as ‘orthotopic’ for these tumors may be inappropriate. A more appropriate model is Ewing sarcoma cells that engineered to express luciferase were implanted into the femur of mice [81], where synergy between PARP inhibitors and chemotherapeutic agents (temozolomide, irinotecan) was demonstrated. However, similar synergy was also reported for five Ewing sarcoma models implanted into the subcutaneous site [31], whereas for another four models there was no synergy. Thus, the value of using a rather labor-intensive and expensive orthotopic model needs to be further justified. For screening, false positives are not necessarily an issue, as secondary testing in an orthotopic model can possibly eliminate these agents. However, false negatives—missing a truly active agent—are a concern. Cilengitide [78] was being active only in the intracranial model but not in the subcutaneous xenograft, for example.

Genetically engineered mouse models (GEMM)

Transgenic models of childhood cancer have been developed over the past 25 years, with the α -amylase-SV40 T-antigen driven osteosarcoma being an early example [82]. Models of solid tumors and leukemias have been constructed and extensively reviewed [83–87]. GEM models have significant advantages over xenograft models for evaluating immune therapies and for understanding the response of tumors in their natural microenvironment. There is an increasing use of GEM models in drug development, particularly for proof of principal type studies. For example, the MMTV-Her2 mammary carcinoma is inhibited by ERBB1/2 small molecule inhibitors, as would be anticipated, and afatinib, an irreversible inhibitor of mutant EGFR, was active in transgenic mouse models with L858/T790M-driven lung cancer [88].

There are, however, less compelling data justifying GEM models of pediatric cancers in drug discovery and development. Various models (neuroblastoma, sarcomas, brain tumors and leukemias) have been engineered. Of these, the most studied is the tyrosine hydroxylase (TH) MYCN neuroblastoma model that mimics poor prognosis MYCN amplified disease in children [89]. Clearly, these models are powerful tools to understand the biology of pediatric cancers. However, their value in identifying novel therapeutics for treatment of these childhood malignancies is at this stage unproven. Perhaps the most studied agent in the TH-MYCN mouse model is the polyamine biosynthesis inhibitor DFMO that retards tumor development when treatment is started early after birth [90]. This model is sensitive to cyclophosphamide, but apparently not to cisplatin, an agent used routinely in the treatment of clinical neuroblastoma. The TH-MYCN model is sensitive to CBL-0137, a novel small molecule that modulates facilitates chromatin transcription (FACT). CBL-0137 results in simultaneous NF- κ B suppression, heat shock factor 1 suppression and p53 activation. CBL-0137 dramatically extended lifespan in this model, whereas it had more limited single-agent activity at dose levels that were highly synergistic with conventional chemotherapeutic agents. Combination of intravenous CBL0137 and cyclophosphamide/topotecan resulted in more than doubling of lifespan against this transgenic neuroblastoma xenograft model [91]; however, this agent showed no significant activity against 5 neuroblastoma xenograft models in PPTP testing, whereas it induced partial or complete regression in 5 of 8 ALL models

[92]. The TH-MYCN mouse model was also sensitive to the BRD4 inhibitor, JQ1, whereas a subcutaneous xenograft (BE(2)-C) was not [93].

Other GEM models of childhood cancer have not been extensively used to identify novel agents. The Sonic hedgehog model of medulloblastoma, driven by *Pcth/p53* mutations that mimic tumors developed in Gorlin syndrome, was instrumental in development of vismodegib for treatment of group 2 medulloblastoma characterized by SHH pathway activation [94]. Other studies have evaluated the EGFR inhibitor erlotinib against heterotopic implants from a transgenic model of alveolar rhabdomyosarcoma that expresses the *Pax3:Fkhr* fusion under control of the *Myf6* promoter in a conditional *p53* null background [95]. While the conclusion was that erlotinib did not have significant activity and that EGFR may not be a high priority target for treatment of alveolar rhabdomyosarcoma [96], however, these tumors showed very diverse growth rates in both treated and control mice, making this a difficult model against which to evaluate therapeutics.

Conclusions

Although human tumor xenografts have significant limitations, our experience with their use for identifying agents that may be fast-tracked for pediatric cancer clinical trials has been relatively successful. The models identify those cytotoxic agents that we know have single-agent activity, and pre-PPTP studies identified topoisomerase I inhibitors, and combinations, as being highly active. These studies have translated well, and some of the combinations are in general use for treatment of childhood tumors. In general, the xenograft studies overpredict for clinical activity, in part because systemic drug exposures in mice exceed those achieved in patients. Consequently, a drug that has albeit robust antitumor activity at the maximum tolerated dose in xenograft models, but little activity below that dose, may not have clinical utility. Examples of such agents are irifolven (MG114) [16], the pre-pro drug PR104 [27] and the aurora kinase inhibitor alisertib [34]. In terms of identifying ‘molecularly’ targeted agents that are cytotoxic (i.e., kill cancer cells), the xenograft models accurately identify agents such as dasatinib, crizotinib and selumetinib that target kinases or pathways activated as a consequence of malignant transformation (*BCR-ABL*, *ALK* and *BRAF*, respectively). Orthotopic models, while conceptually appealing, have not necessarily offered an advantage in terms of identifying agents with significant activity, or shown greater predictive value for successful translation into the clinic. For brain tumors, it would be anticipated that intracranial models may better reflect clinical responses due to poor drug penetration if there is a patent blood–brain barrier. The value of genetically engineered models remains to be validated for drug screening, although they are clearly important for understanding tumor biology, immunotherapy and microenvironmental interactions. Thus, the choice of model may depend upon the particular experimental objective.

One additional issue that may limit the value of these models in accurately translating to clinical efficacy is illustrated for the MDM2 inhibitors, where the inhibitory molecules are optimized for human proteins and hence may not reveal the toxic effects on murine tissues but maintain high-level activity in these preclinical models [54]. For these agents, therapeutic index in preclinical models may no longer be applicable.

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Drug	Ewing Sarcoma					Rhabdomyosarcoma							
	SKNEP 1	EW-5	EW-8	TC- 71	CHLA- 258	Rh10	Rh28	Rh30	Rh30R	Rh41	Rh65	Rh18	Rh36
Vincristine													
Eribulin													
BAL101553													
Cabazitaxel													
Docetaxel													
Abraxane													

	Progressive Disease 1 (PD1)
	Progressive Disease 2 (PD2)
	Stable Disease (SD)
	Partial Response (PR)
	Complete Response (CR)
	Maintained Complete Response (MCR)

Fig. 1.

Comparison of the antitumor activity of tubulin-interacting drugs in pediatric soft tissue sarcoma xenograft models. New anti-tubulin agents are compared with the standard of care agent, vincristine. Data are from references [9, 22–24, 97]

Fig. 2.

Combination of the PARP inhibitor, talazoparib, is synergistic with temozolomide in some Ewing sarcoma xenograft models. Anti-tumor activity of temozolomide (30 mg/kg for 5 days), talazoparib (0.25 mg/kg twice daily for 5 days) or in combination against 'sensitive' Ewing sarcoma models. *Combination A* (temozolomide 30 mg/kg for 5 days; talazoparib 0.1 mg/kg twice daily for 5 days) and *combination B* (temozolomide 12 mg/kg for 5 days; talazoparib 0.25 mg/kg twice daily for 5 days). Graphs show growth of individual tumors in SCID mice. Data from [31] with permission

Fig. 3. Selinexor in vivo objective response activity. **a** Solid tumor and brain tumor models. KT-10 (Wilms tumor). BT-50 (medulloblastoma; group B [98]), BT-41 (ependymoma) and BT-45 (medulloblastoma; group A [98]). **b** Acute lymphoblastic leukemia models ALL-8 (primary T cell ALL) and ALL-19 (primary B-precursor ALL). Kaplan–Meier curves for EFS (*left*), median relative tumor volume graphs (*center*) and individual tumor volume graphs (*right*) are shown. Treated (*black lines*), statistical significance (*p* values) of the difference between treated and control groups are included. Data from [61], with permission