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Pharmacodynamic and genomic markers associated with response to the XPO1/CRM1 inhibitor selinexor (KPT-330): a report from the Pediatric Preclinical Testing Program

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Abstract

Background—Selinexor (KPT-330) is an inhibitor of the major nuclear export receptor, exportin 1 (XPO1, also termed chromosome region maintenance 1, CRM1) that has demonstrated activity in preclinical models and clinical activity against several solid and hematological cancers.

Procedures—Selinexor was tested against the Pediatric Preclinical Testing Program (PPTP) *in vitro* cell line panel at concentrations from 1.0 nM to 10 μM and against the PPTP *in vivo* xenograft panels administered orally at a dose of 10 mg/kg thrice weekly for 4 weeks.

Results—Selinexor demonstrated cytotoxic activity *in vitro*, with a median relative IC₅₀ value of 123 nM, (range 13.0 nM to >10 μM). Selinexor induced significant differences in event-free survival (EFS) distribution in 29 of 38 (76%) of the evaluable solid tumor xenografts and in 5 of 8

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(63%) of the evaluable ALL xenografts. Objective responses (partial or complete responses, PR/CR) were observed for 4 of 38 solid tumor xenografts including Wilms tumor, medulloblastoma (n=2) and ependymoma models. For the ALL panel, 2 of 8 (25%) xenografts achieved either CR or maintained CR. Two responding xenografts had *FBXW7* mutations at R465 and two had *SMARCA4* mutations. Selinexor induced p53, p21 and cleaved PARP in several solid tumor models.

Conclusions—Selinexor induced regression against several solid tumor and ALL xenografts and slowed tumor growth in a larger number of models. Pharmacodynamic effects for XPO1 inhibition were noted. Defining the relationship between selinexor systemic exposures in mice and humans will be important in assessing the clinical relevance of these results.

Keywords

Preclinical Testing; Developmental Therapeutics; XPO1 inhibitor

INTRODUCTION

Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (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) [1,2]. This complex is transported through the nuclear pore complex (NPC) to the cytoplasm, and in the cytoplasm RanGTP is hydrolyzed to RanGDP releasing XPO1 and the cargo protein, with XPO1 then recirculating to the nucleus. XPO1 is involved in the nuclear export of over 200 NES-containing proteins, with many of them being tumor suppressor proteins and cell cycle regulators [2]. Consistent with a role for XPO1 in cancer, XPO1 is upregulated in a range of hematologic and solid tumors, and overexpression is associated with poor prognosis [3–6]. Because alterations in nuclear-cytoplasmic trafficking may lead to the cytoplasmic mislocalization of proteins involved in cell cycle, cell survival or tumor suppression, forcing their nuclear localization may have potential as a cancer therapeutics strategy [2].

Leptomycin B (elactocin) was the first recognized inhibitor of XPO1. It inhibits the export activities of XPO1 by forming a covalent adduct with cysteine-528 of XPO1 [7–9]. Covalent adducts at cysteine-528 functionally inactivate XPO1 and target it for proteasomal degradation [8] Prior to its identification as an inhibitor of XPO1, leptomycin B had been studied as an anticancer agent and showed in vitro and in vivo activity against a range of cancer cell lines [10,11]. However, leptomycin B produced profound vomiting, anorexia and malaise when administered to patients in a phase 1 clinical trial, which led to the agent being dropped from clinical development [12]. Subsequently, SINE compounds have been developed that also work through formation of a covalent bond to cysteine-528 of XPO1 [13], but that have improved tolerability while maintaining anticancer activity [14].

SINE compounds by inhibiting XPO1 are able to force nuclear retention and activation of multiple proteins involved in cell cycle regulation including p53, FOXO, p21, Rb, p27 and I κ B [2]. Preclinical activity for SINE compounds have been documented against adult solid

tumors [2], as well as for hematological malignancies including T-cell acute lymphoblastic leukemia (ALL) [15], acute myeloid leukemia (AML) [15,16], multiple myeloma [17,18], and non-Hodgkin lymphoma (NHL) [19,20]. As an example, verdinexor (KPT-335) potently inhibits human and canine lymphoma cell lines with low nanomolar potency, and in a phase 1 study with dose expansion in dogs with lymphoma, partial responses were noted in 4 of 20 treated animals [21]. In a subsequent phase 2 trial of verdinexor for canine lymphoma, objective responses (PR or CR) were noted in 20 of 58 (34%) treated animals [22].

Selinexor is currently in early phase clinical trials in both adult and pediatric malignancies. Phase 1 testing in adults evaluated either twice or thrice weekly dosing, with single agent phase 2 evaluations going forward primarily using the twice-weekly schedule [23,24]. The primary and most prominent adverse events are fatigue, anorexia, nausea and in solid cancer patients also thrombocytopenia. Responses were noted in the phase 1 studies for several diagnoses. For example, for adults with AML approximately 10% of patients achieved a complete response, and a randomized phase 2 study comparing selinexor to physician's choice of standard treatment is ongoing for adults 60 years old with relapsed/refractory AML who are ineligible for intensive chemotherapy and/or transplantation (NCT02088541). In a phase 2 evaluation of selinexor, 37% of patients (19 of 52) with heavily pretreated NHL achieved either a partial or complete response [25].

Because of its novel mechanism of action, selinexor was tested against the entire *in vitro* and *in vivo* panels of pediatric tumor models.

MATERIALS AND METHODS

In vitro testing

Testing was performed using DIMSCAN [20], as previously described in a characterized panel of 23 cell lines [26]. Cells were incubated in the presence of selinexor for 96 hours at concentrations from 1.0 nM to 10 μ M and analyzed as previously described [27].

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [28]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [29]. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Eight to ten mice were used in each control or treatment group. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [%hCD45+] cells [ALL xenografts] were determined and responses were determined using three activity measures as previously described [28]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

Selinexor was provided to the Pediatric Preclinical Testing Program by Karyopharm Therapeutics Inc. (Newton, MA), through the Cancer Therapy Evaluation Program (NCI). Selinexor was formulated in 0.56% w/v Pluronic F-68/0.56% w/v Plasdone **K-29/32** water-soluble polyvinylpyrrolidone and stored for up to 7 days at 4°C, protected from light. Selinexor was administered orally at 10 mg/kg 3 times per week (Monday/Wednesday/Friday) for 4 consecutive weeks with an additional 2 weeks of observation. Selinexor was provided to each consortium investigator in coded vials for blinded testing.

Pharmacodynamic studies

KT-10 tumors that regress rapidly after treatment with selinexor, were harvested 24 hours after a single dose of drug (10 mg/kg). Other, less responsive tumors, were harvested 2 hours after dose 6 (MWF dosing) at 10 mg/kg/dose. Tumors were processed for immunoblotting as previously described [30]. Immunoblots were probed for p53, p21, PARP and cleaved PARP and XPO1/CRM1. Three control and three treated tumors were analyzed for each xenograft line. GAPDH was used as a loading control. KT-10, KT-11, SK-NEP-1, CHLA258, Rh28 and Rh30 tumors were fixed in 10% buffered formalin and paraffin sections were analyzed by H&E as well with the following antibodies: FOXO1 (#2880, Cell Signaling), IKB (ab32518, Abcam), NFkB (sc-8008, Santa Cruz), pRb-phos (ab76298, Abcam), Mcl-1 (sc-819, Santa Cruz), β -catenin (#610154 BD), ERK-phos (ab32538, Abcam), survivin (ab24479, Abcam), and p53 (sc-126, Santa Cruz).

RESULTS

In vitro testing

Selinexor was tested against the PPTP's in vitro cell line panel at concentrations ranging from 1.0 nM to 10.0 μ M using the PPTP's standard 96 hour exposure period. The median relative IC₅₀ (rIC₅₀) value for the cell line panel was 123 nM, with a range from 13 nM (CHLA-9, Ewing sarcoma) to > 10 μ M (Rh18 rhabdomyosarcoma). A metric used to compare the relative responsiveness of these cell lines to selinexor is the ratio of the median rIC₅₀ of the entire panel to that of each cell line (Table I and Figure 1A). Higher ratios are indicative of greater sensitivity to selinexor by bars to the right of the midpoint line. As shown in Figure 1A, the cell lines of the Ewing sarcoma panel (bars with stripes) were relatively sensitive to selinexor with 3 of 4 cell lines having rIC₅₀ values less than the median for the entire panel. The ALL cell lines (bars with dots) include 3 lines that were less sensitive than the panel median and 2 that were more sensitive. Both rhabdoid tumor cell lines (BT-12 and CHLA-266) had rIC₅₀ values greater than then panel median. The Relative In/Out (I/O)% values compare the relative difference in final cell number compared with the

starting cell number for treated cells and for control cells. Relative I/O% values ranged between 100% (no treatment effect) to -100% (complete cytotoxic effect), with a Relative I/O% value of 0% being observed for a completely effective cytostatic agent. Figure 1B shows that most cell lines had Relative I/O% values between -75% and -100%, consistent with a prominent cytotoxic effect. Rh18 showed clear resistance to selinexor. Both the rIC₅₀ values and the Y_{min} values were similar for p53 mutated and wild-type cell lines, with no statistically significant differences for either parameter based on p53 status.

In vivo testing

Selinexor was tested against the PPTP solid tumor xenografts using oral dosing on a thrice weekly (M-W-F) for 4 weeks schedule. The total planned treatment and observation period was 6 weeks. Toxicity testing using cohorts of 5 non-tumored animals was performed prior to efficacy testing. Doses of 15 mg/kg and greater were not tolerated with treatment stopped within two weeks of initiation because of excessive toxicity. The 10 mg/kg dose was adequately tolerated with a maximum weight loss of approximately 10% with recovery during continued dosing, and this dose was selected for efficacy testing. Treatment at the 10 mg/kg dose resulted in 4 toxicity related deaths of 450 mice (0.9%), whereas there were no deaths in the control groups (0/441).

All 46 tested xenograft models were considered evaluable for efficacy, with one ALL model (ALL-19) having to be repeated because of excessive numbers of mice developing lymphomas. Complete details of testing are provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Selinexor induced significant differences in EFS distribution compared to control in 29 of 38 (76%) solid tumor xenografts and in 5 of 8 (63%) ALL xenografts (Table II). For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume. Selinexor induced tumor growth inhibition meeting criteria for intermediate (n=10) or high (n=1) EFS T/C activity in 11 of 32 (34%) solid tumor xenografts evaluable for this metric. Intermediate or high activity for the EFS T/C metric occurred most frequently in the Wilms tumor panel (2 of 3) and the Ewing sarcoma panel (4 of 5), but was observed for only 1 of 6 rhabdomyosarcoma xenografts, 1 of 6 neuroblastoma xenografts and for no osteosarcoma xenografts (n=6). Intermediate or high EFS T/C activity was also observed for the rhabdoid panel (n=1), medulloblastoma (n=2) and glioblastoma panel (1 of 4). For the ALL panel, 2 of 8 (38%) xenografts met criteria for intermediate or high EFS T/C activity.

Objective responses were observed in 4 of 38 (11%) solid tumor xenografts, including a maintained complete response (MCR) for the Wilms tumor xenograft KT10, a PR and CR for the medulloblastoma xenografts BT-45 and BT-50, respectively, and a CR for the slow-growing ependymoma xenograft BT-41 (Figure 2A). As many control BT-41 and BT-45 tumors did not reach event status (4-fold increase in tumor size) during the 6 week

evaluation period, the treatment groups for these models were not statistically different from controls in terms of EFS distribution. For the ALL panel, 2 of 8 (25%) xenografts achieved either CR (T-cell ALL xenograft, ALL-8) or MCR (B-precursor ALL xenograft, ALL-19) (Figure 2B). The objective response results for both solid tumor and leukemia models are represented using a 'COMPARE' format, based on the objective response scoring criteria centered around the midpoint score of 0 that represents stable disease, and in heat-map format (Figure 3).

Selinexor induced very rapid regression of KT-10 Wilms tumor xenografts, but was less active against other solid tumors. To determine whether selinexor was inducing pharmacodynamics effects associated with XPO1/CRM1 inhibition (increased p53 and cleavage of PARP), KT-10 tumors were harvested 24 hours after dose 1, whereas the other tumors were harvested 2 hours post dose six. As shown in Figure 4A, selinexor induced a robust increase in p53 in KT-10 xenografts with cleavage of PARP apparent after the first dose of drug, consistent with the rapid response of this tumor to treatment. Of interest, however, was that the basal expression levels of p21 are relatively high and there was very little increase in p21 expression following selinexor treatment. In tumors that responded poorly [KT-11 (PD1) and SKNEP1, CHLA-258, Rh28, Rh30 (all PD2)] a slight increase in p53 was detected only for Rh28 and Rh30 xenografts. However, p21 was induced by selinexor in CHLA-258, Rh28 and Rh30 tumors. PARP cleavage was clearly induced by treatment in Rh30 tumors, but was only slightly increased in Rh28 and CHLA258 xenografts (Figure 4B).

While the Wilms tumor xenograft KT-10 responded better than other sarcomas to the cytotoxic and the cytostatic effects of selinexor, analysis of sections demonstrated effects of response in all the tumor types. Comparison of sections from all the tumors, pre- and post-treatment showed increased areas of necrosis, decreased cell number and increased exposure of tumor stromal cells. Interestingly those were very prominent in Rh28, the alveolar rhabdomyosarcoma tumor that showed poor response at the end of the 4 week in vivo study (Supplemental Figure 1).

Immunohistochemical analysis of sections from the most sensitive tumor, KT-10 (Wilms tumor), demonstrated the pharmacodynamic marker changes that were expected upon XPO1 inhibition (Figure 4C). These included the accumulation of XPO1 protein cargos in the nucleus: FOXO1, IKB, NFkB, pRb, ERK and survivin. As expected, selinexor not only induced nuclear accumulation of pRb, but also reduced its inactive form (i.e. decreased phosphorylated pRb), suggesting reduced proliferation. The treatment also induced the nuclear accumulation of phosphorylated ERK. In addition to effects on XPO1 cargos, selinexor also reduced the expression of the anti-apoptotic protein Mcl-1 and β -catenin that transduce pro survival signals and induce cell cycle promotion.

Analysis of the models that were less sensitive to the cytostatic and cytotoxic effects of selinexor (KT-11, SK-NEP-1, CHLA258, Rh28 and Rh30), showed less pronounced changes in some of the above pharmacodynamics markers for XPO1 inhibition. For example, Rh28 showed partial FOXO3 nuclear accumulation with much cytoplasmic stain.

In addition it showed minimal reduction of β -catenin and almost no reduction in Mcl-1 protein (Supplemental Figure 2).

Surprisingly, analysis of the p53 nuclear localization in KT-10, the most selinexor sensitive tumor on the study did not show increased nuclear accumulation following treatment (not shown). KT-10 tumor cells express a non-clinically important p53 mutation (R290H) with wild type transcriptional activity [31], and therefore p53 would be expected to be enriched in the nucleus in the treated cells. However, p53 proteins, either wild type in CHLA258 and Rh28 tumors or mutant in Rh30 tumors were sensitive to XPO1 inhibition and showed nuclear accumulation upon selinexor treatment in these three cases (Supplemental Figure 3). This experiment suggests that p53 nuclear accumulation is a pharmacodynamic marker of XPO1 inhibition only in certain cells types.

The mutation profiles for models with objective responses for which exome sequencing data were available (KT-10, BT-50, ALL-8, and ALL-19) were examined (Supplemental Table II). All mutations were verified and assessed as somatic using a virtual normal subtraction algorithm. Recurring mutations were observed for *FBXW7* and *SMARCA4*. For *FBXW7*, known oncogenic mutations were identified for both BT-50 (R465H) and ALL-8 (R465C). One additional tested xenograft (ALL-31) had an *FBXW7* mutation, but it was in the N-terminal region in the nuclear localization signal domain (c.45_46insCCT; p.15_16TR>TLR). For *SMARCA4*, the R1189Q mutation, which is predicted to be deleterious by both SIFT and PolyPhen, was observed in BT-45 and ALL-8. KT-10 has a *PALB2* mutation that leads to defective homologous recombination and to sensitivity to PARP inhibition [32].

DISCUSSION

Similar to the natural inhibitors of XPO1 (e.g. leptomycin B), the molecularly modeled SINE selinexor has activity against cancer cells both *in vitro* and *in vivo*. However, the more specific inhibitors such as selinexor do not show the profound toxicities of the early compounds. The most common adverse events in the first phase 1 trials of selinexor in adults were thrombocytopenia, nausea, anorexia, and fatigue. Early clinical experience with selinexor has demonstrated that objective responses can be induced at doses and schedules of the agent that are generally well tolerated [23,24,33].

The PPTP *in vitro* results revealed a broad range of activity against pediatric cancer types. All but two of the cell lines tested had IC₅₀ values between 13-317 nM. Relative to the entire panel, the Ewing sarcoma and rhabdoid tumor cell lines were somewhat more sensitive than the remaining cell lines, but the differences in sensitivity were not large. Across the entire panel, selinexor showed a prominent cytotoxic effect independent of TP53 mutation status, indicating the lack of dependence of the agent on an intact p53 pathway for activity.

At the 10 mg/kg thrice weekly dose selected for study, selinexor showed broad tumor growth inhibitory activity against the solid tumor xenografts, with statistically significant differences in EFS distribution in 76% of xenografts. Growth delay was most pronounced

for the Ewing sarcoma xenografts and least apparent for the osteosarcoma xenografts. In terms of objective responses, these were seen in Wilms tumor (one maintained complete response), medulloblastoma, and ependymoma. The responding Wilms tumor xenograft (KT-10) is known to be defective in homologous recombination as a result of biallelic *PALB2* mutation [32]. KT-10 and the responding medulloblastoma xenograft BT-45 were the two PPTP solid tumor xenografts that showed objective responses to both the PARP inhibitor talazoparib and to cisplatin [32,34]. For the ALL panel, CRs were noted for 2 of 8 models, consistent with prior preclinical work on ALL and with the clinical activity observed for selinexor against lymphoid malignancies [15,23]. It is possible that even higher levels of activity are achievable with changes to the dosing and schedule of the drug. A twice weekly schedule and caloric supplementation may allow for increased drug exposures while minimizing toxicity.

While most PPTP xenografts failed to show objective responses, several showed pronounced sensitivity to selinexor with CRs observed. These models may be considered “exceptional responders” and, analogous to reports describing exceptional clinical activity for other targeted agents, may show specific genomic lesions that lead to sensitivity [35,36]. Regarding the xenografts exhibiting objective responses, the Wilms tumor xenograft (KT-10) with the most pronounced solid tumor response is known to be defective in homologous recombination as a result of biallelic *PALB2* mutation [32]. Evaluations of additional models will be required to determine whether defective homologous recombination in general (or specific to *PALB2* loss of function) sensitizes to SINE compounds. The identification of mutated *PALB2* in sensitive selinexor tumors is potentially more interesting in light of the recent report that SINE compounds inhibit the expression of DNA damage repair proteins [37]. Finding of activity or minimal activity of selinexor across many sarcoma tumor types together with this connection to DNA damage repair suggest that combination treatment of DNA damage inducing agents with selinexor could potentially be effective treatment in sarcoma tumors, an hypothesis that warrants further preclinical testing.

The only two models tested that harbor *FBXW7* “hot spot” mutations showed objective responses. These hot spot mutations occur in the WD40 substrate-binding domain of *FBXW7* [38]. *FBXW7* encodes three transcripts (α , β , and γ) that result from alternative splicing, with the transcripts showing distinctive cellular localization [39]. The *FBXW7* α isoform localizes to the nucleus and is responsible for targeting a number of growth/survival promoting factors for degradation [38,40,41]. *FBXW7* with hot spot mutations acts in a dominant-negative fashion, such that when mutations are heterozygous (as for BT-50 and ALL-8) they exert a marked effect on levels of *FBXW7* target proteins as a result of their inhibition of wildtype *FBXW7* in heterodimers [38]. SINE compounds have been shown to enhance nuclear localization of *FBXW7* in pancreatic cancer cells [42], and it is plausible that in xenografts with *FBXW7* hot spot mutations they are able to elevate the level of active wildtype homodimers sufficiently so that target oncogenic proteins are degraded leading to anticancer activity. ALL-31, which did not respond to selinexor, also harbors an *FBXW7* genomic alteration: a 3-base insertion in the N-terminal region that is known to disrupt the nuclear localization signal of *FBXW7* and that has previously been reported in prostate cancer and Wilms tumor [38,43]. A consequence of this mutation is the cytoplasmic, rather

than nuclear, localization of FBXW7 α [38,44]. Hence, the cellular effects and response to SINE compounds for cancer cells with this mutation are likely distinctive from the effects observed for cells with *FBXW7* mutations in the WD40 substrate-binding domain. Further testing will be required to define the relationship between *FBXW7* hot spot mutations and sensitivity to SINE compounds.

A second gene that was recurrently mutated among responsive xenografts was *SMARCA4*, a gene that encodes an ATP-dependent catalytic subunit of SWI-SNF complexes [45]. *SMARCA4* mutations were present in a medulloblastoma xenograft (BT-45) and in a T-cell ALL xenograft (ALL-8). Germline mutations in *SMARCA4* predispose to rhabdoid tumor and to small cell carcinoma of the ovary, hypercalcemic type (malignant rhabdoid tumor of the ovary) [45]. Somatic mutations of *SMARCA4* have been reported at relatively low frequency in a number of cancers, including medulloblastoma, mantle cell lymphoma, Burkitt lymphoma, T-cell ALL, and others [45–48].

Pharmacodynamic changes induced by selinexor were examined in six xenografts including the KT-10 Wilms tumor that showed rapid regression. Pharmacodynamic effects were documented using the 10 mg/kg dose, with protein levels of p53 and p21 increased in some, but not all, tumors; similarly, PARP cleavage was induced in three of the studied tumors. This varied response underlines the broad range of effects that result from nuclear export inhibition. Given the number of proteins shuttled out of the nucleus by XPO1, it is reasonable to speculate that multiple pathways will be up- or down-regulated by selinexor. While nuclear localization of XPO1 cargos are good pharmacodynamic markers, demonstrating drug bioavailability, some of these markers may serve as markers of response. In the most sensitive KT-10 tumors, FOXO1, IKB, NFkB, pRb, ERK and survivin were locked in the nuclei of selinexor treated tumor cells. This included phosphorylated ERK, which is associated with growth suppression signals [49]. Some of these markers revealed the cytostatic and cytotoxic effects of selinexor, showing reduction in phosphorylated pRb and decrease of the anti-apoptotic protein Mcl-1 as well as the proto-oncogene β -catenin. Interestingly, selinexor non-sensitive sarcoma tumors showed less significant changes in these pharmacodynamics markers. Since these observations are seen one to two weeks after treatment initiation, they may potentially serve as early predictive markers of response. Unlike the above markers, p53 nuclear localization serves as pharmacodynamic marker only in some sarcomas.

As with other agents, the ability for these murine data to translate accurately to the clinic depends upon drug exposures being similar in the models at doses causing tumor regressions to exposures achieved in patients. Mice in our experiments were dosed with an oral suspension of 10 mg/kg selinexor (KPT-330) three times a week. At this dose and regimen, selinexor was shown to be efficacious and well tolerated in various preclinical cancer models including those reported in this manuscript. With a single oral administration of selinexor at 10 mg/kg (30 mg/m²) in CD1 mouse, an AUC_{inf} of 9530 hr*ng/mL and a C_{max} of 1,240 ng/mL (~2.8 μ M) were observed (Karyopharm Therapeutics, unpublished data). Thus, the exposure to drug in CD1 mice is similar to that determined in patients treated with 65 mg/m² [50]. Poor response of a certain xenograft models in this study to selinexor is probably not because of poor drug penetration, but more likely due to the genetic

background of the specific cancer models, as discussed above. Evidence for this is the nuclear localization of either p53 or other XPO1 cargos observed in all the tumors following selinexor treatment, irrespective of response. This nuclear localization is a good marker for drug exposure. Thus, as with other targeted therapies, inhibition of ‘target’ does not necessarily correlate with cellular outcome. MDM2 inhibitors [51] or kinase inhibitors being other examples [52–54].

These data support the further development of selinexor for pediatric malignancies. Defining the relationship between selinexor systemic exposures in mice and humans will be important in better assessing the clinical relevance of these results. The first pediatric phase 1 trial for lymphoid malignancies is open (NCT02091245), and a phase 1 trial for children with solid tumors is under development. Additional data from adult clinical trials and further preclinical testing are needed to clearly define a responder hypothesis and to discover rational drug combinations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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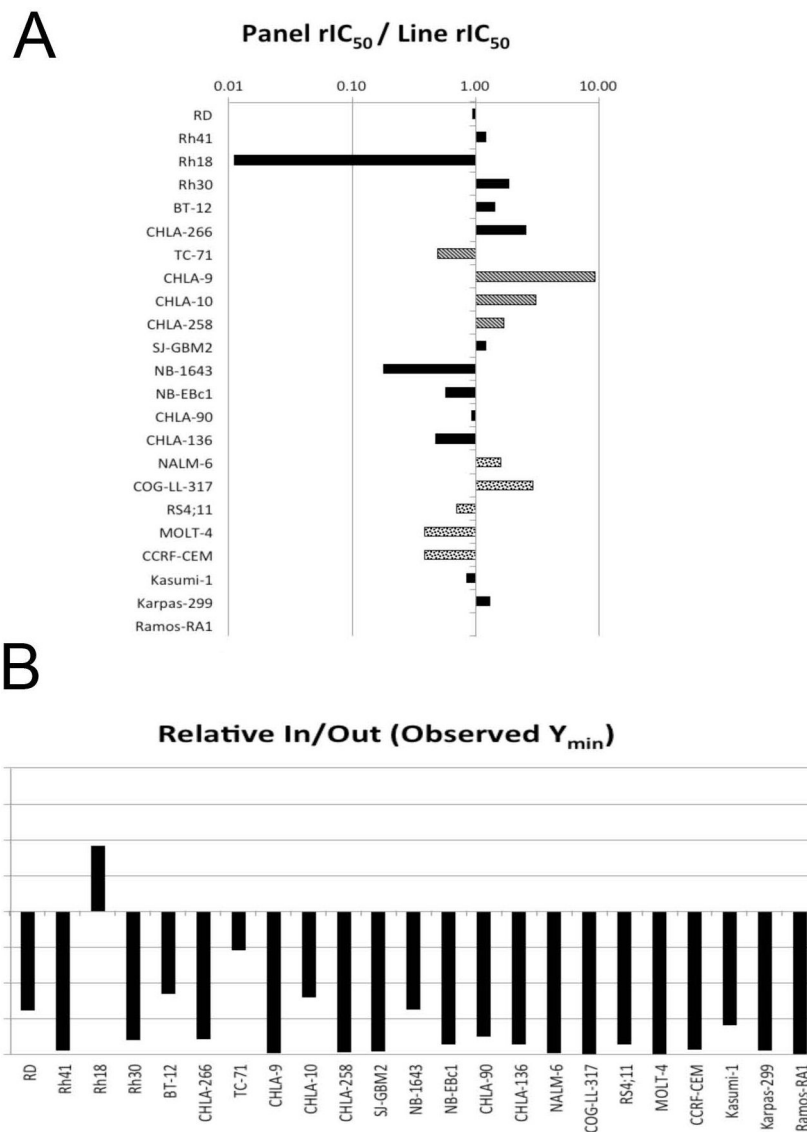


Figure 1. Selinexor *in vitro* activity: A. The median rIC_{50} ratio graph shows the relative rIC_{50} values for the cell lines of the PPTP panel. Bars with stripes represent Ewing sarcoma cell lines and bars with dots represent ALL cell lines. Each bar represents the ratio of the panel rIC_{50} to the rIC_{50} value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity; B. Most cell lines have Relative I/O% values between -75% and -100% , consistent with a prominent cytotoxic effect. Rh18 shows clear resistance to selinexor.

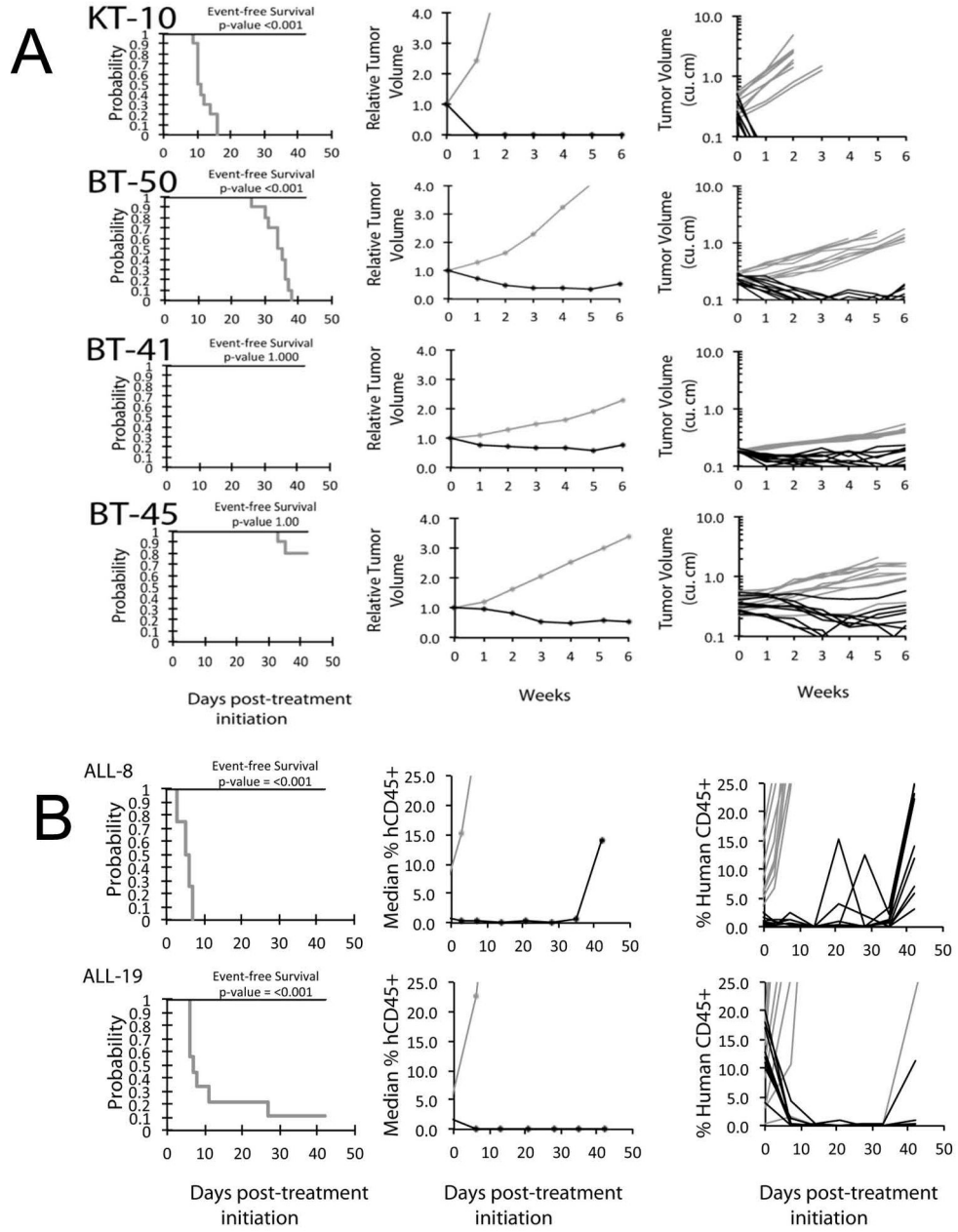


Figure 2. Selinexor *in vivo* objective response activity. A solid tumor and brain tumor models. KT-10 (Wilms tumor). BT-50 (medulloblastoma), BT-41 (ependymoma), and BT-45 (medulloblastoma); B ALL models, ALL-8 (T-cell), ALL-19 (B precursor): Kaplan-Meier curves for EFS (left), median relative tumor 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.

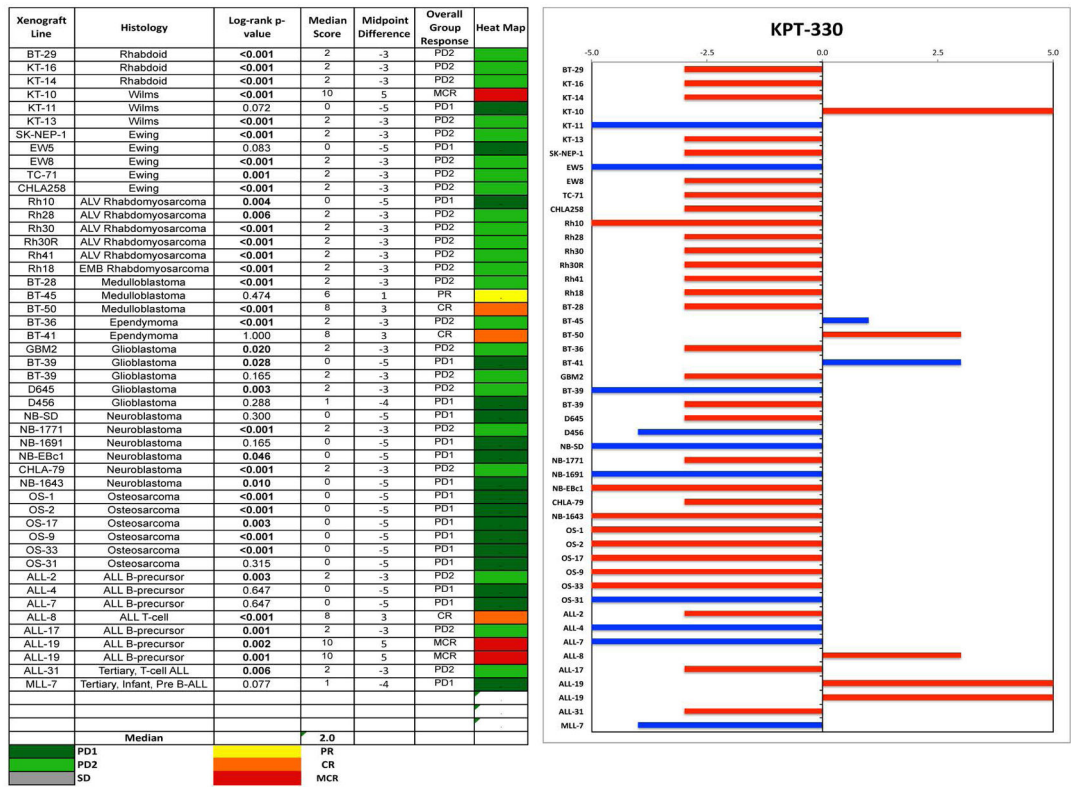


Figure 3.

Left: The colored heat map depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of 2 but <6, and low activity by a score of <2. Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

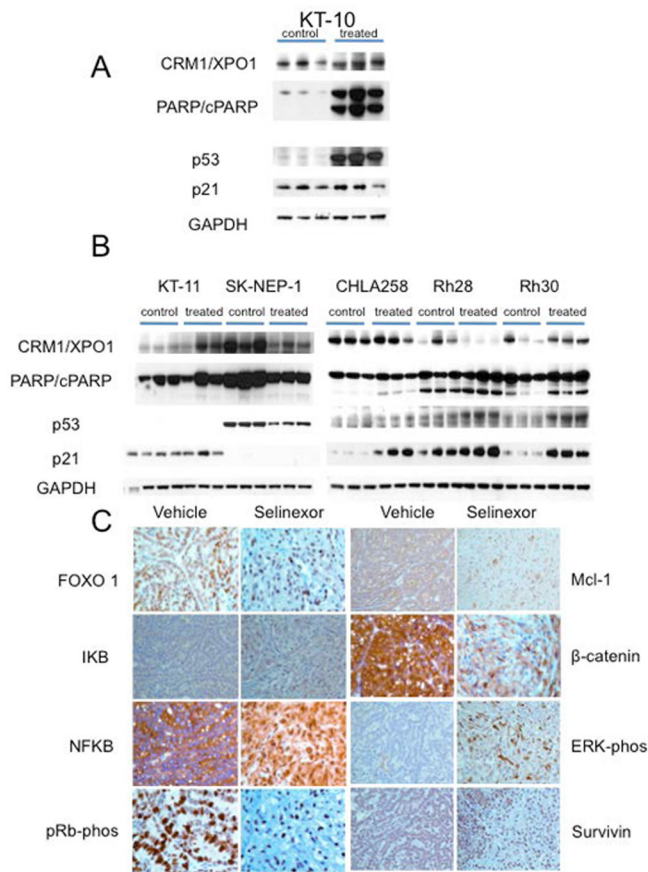


Figure 4.

A. Pharmacodynamic changes induced by selinexor. A. KT-10 Wilms tumor. Tissue was harvested 24 hr after a single dose of selinexor (10 mg/kg); B. Tumors were harvested 2 hr after dose six of selinexor (10 mg/kg/dose). With the exception of SK-NEP1, all tumors are p53 wild type by sequence analysis. Three control and 3 tumors from treated mice were used for each xenograft line. GAPDH was used as a loading control. **B.** In vivo treatment with selinexor blocked XPO1 cargos in the nucleus and reduced the expression of signaling proteins that are associated with cell proliferation. Sections from vehicle and selinexor treated KT-10 were analyzed by immunohistochemistry (IHC). Cells from tumors that were treated with selinexor show increased nuclear accumulation of FOXO1, IKB, NFKB, pRb, ERK and Survivin. In addition the IHC slides show reduction in Mcl-1 and β-catenin.

Table 1

In vitro activity of selinexor against PPTP cell lines.

Cell Line	Histotype	rIC ₅₀ (μM)	Panel rIC ₅₀ / Line rIC ₅₀	Ymin % (Observed)	P53 Status
RD	Rhabdomyosarcoma	0.128	0.96	1.7	Mutant
Rb41	Rhabdomyosarcoma	0.100	1.22	0.6	Mutant
Rh18	Rhabdomyosarcoma	>10.000	<0.01	69.8	WT
Rh30	Rhabdomyosarcoma	0.065	1.88	1.7	Mutant
BT-12	Rhabdoid	0.085	1.44	3.5	WT
CHLA-266	Rhabdoid	0.048	2.56	2.8	WT
TC-71	Ewing sarcoma	0.247	0.50	0.9	Mutant
CHLA-9	Ewing sarcoma	0.013	9.32	0.0	WT
CHLA-10	Ewing sarcoma	0.040	3.06	2.5	Mutant
CHLA-258	Ewing sarcoma	0.073	1.68	0.5	WT
SJ-GBM2	Glioblastoma	0.101	1.21	0.2	Mutant
NB-1643	Neuroblastoma	0.683	0.18	6.6	WT
NB-EBc1	Neuroblastoma	0.214	0.57	1.6	WT
CHLA-90	Neuroblastoma	0.131	0.94	3.4	Mutant
CHLA-136	Neuroblastoma	0.255	0.48	2.0	WT
NALM-6	ALL	0.077	1.59	0.0	WT
COG-LL-317	ALL	0.042	2.90	0.0	WT
RS4;11	ALL	0.174	0.71	1.1	WT
MOLT-4	ALL	0.317	0.39	0.0	WT
CCRF-CEM (1)	ALL	0.317	0.39	0.2	Mutant
Kasumi-1	AML	0.143	0.86	5.8	Mutant
Karpas-299	ALCL	0.093	1.31	0.2	Mutant
Ramos-RA1	NHL	0.123	1.00	0.0	Mutant
Median		0.123	1.00	1.1	
Minimum		0.013	<0.01	0.0	
Maximum		>10.000	9.32	69.8	

Table II

Summary of *in Vivo* Activity of selinexor.

Line	Tumor Type	Estimate of Median Time to Event	P-value	EFST/C	Median RTV/CD45 at End of Study	Tumor Volume T/C	Median Group Response	EFS Activity
BT-29	Rhabdoid	> EP	<0.001	> 1.8	1.5	0.29	PD2	NE
KT-16	Rhabdoid	34.8	<0.001	4.1	>4	0.39	PD2	Int
KT-14	Rhabdoid	> EP	<0.001	> 1.7	2.3	0.48	PD2	NE
KT-10	Wilms	> EP	<0.001	> 4.0	0.0	0.00	MCR	High
KT-11	Wilms	20.2	0.072	1.3	>4	0.69	PD1	Low
KT-13	Wilms	18.0	<0.001	2.0	>4	0.35	PD2	Int
SK-NEP-1	Ewing	36.2	<0.001	3.9	>4	0.34	PD2	Int
EW5	Ewing	9.9	0.083	1.1	>4	0.83	PD1	Low
EW8	Ewing	22.1	<0.001	2.7	>4	0.67	PD2	Int
TC-71	Ewing	25.0	0.001	3.1	>4	0.79	PD2	Int
CHLA258	Ewing	31.8	<0.001	3.3	>4	0.22	PD2	Int
Rh10	Alveolar RMS	34.5	0.004	1.4	>4	0.55	PD1	Low
Rh28	Alveolar RMS	32.4	0.006	1.5	>4	0.48	PD2	Low
Rh30	Alveolar RMS	28.4	<0.001	3.2	>4	0.55	PD2	Int
Rh30R	Alveolar RMS	24.2	<0.001	1.7	>4	0.42	PD2	Low
Rh41	Alveolar RMS	26.2	<0.001	1.9	>4	0.44	PD2	Low
Rh18	Embryonal RMS	11.1	<0.001	1.6	>4	0.78	PD2	Low
BT-28	Medulloblastoma	30.8	<0.001	3.1	>4	0.69	PD2	Int
BT-45	Medulloblastoma	> EP	0.47	NE	0.6	0.28	PR	NE
BT-50	Medulloblastoma	> EP	<0.001	> 1.2	0.5	0.17	CR	NE
BT-36	Ependymoma	> EP	<0.001	> 1.1	1.6	0.37	PD2	NE
BT-41	Ependymoma	> EP	1.000	.	0.7	0.49	CR	NE
GBM2	Glioblastoma	28.5	0.020	2.2	>4	0.62	PD2	Int
BT-39	Glioblastoma	17.4	0.165	2.0	>4	0.70	PD2	Low
D645	Glioblastoma	11.4	0.003	2.0	>4	0.44	PD2	Low
D456	Glioblastoma	9.0	0.288	1.5	>4	0.60	PD1	Low

Line	Tumor Type	Estimate of Median Time to Event	P-value	EFST/C	Median RTV/CD45 at End of Study	Tumor Volume T/C	Median Group Response	EFS Activity
NB-SD	Neuroblastoma	10.5	0.300	1.1	>4	0.97	PD1	Low
NB-1771	Neuroblastoma	20.8	<0.001	2.0	>4	0.44	PD2	Low
NB-1691	Neuroblastoma	6.6	0.165	1.2	>4	0.80	PD1	Low
NB-EBc1	Neuroblastoma	7.7	0.046	1.4	>4	0.64	PD1	Low
CHLA-79	Neuroblastoma	39.1	<0.001	4.3	>4	0.58	PD2	Int
NB-1643	Neuroblastoma	6.7	0.010	1.2	>4	0.64	PD1	Low
OS-1	Osteosarcoma	30.4	<0.001	1.3	>4	0.57	PD1	Low
OS-2	Osteosarcoma	18.0	<0.001	1.1	>4	0.91	PD1	Low
OS-17	Osteosarcoma	26.5	0.003	1.4	>4	0.74	PD1	Low
OS-9	Osteosarcoma	30.5	<0.001	1.3	>4	0.73	PD1	Low
OS-33	Osteosarcoma	18.3	<0.001	1.4	>4	0.71	PD1	Low
OS-31	Osteosarcoma	17.6	0.315	1.0	>4	0.93	PD1	Low
ALL-2	ALL B-precursor	36.8	0.003	1.9	>25	.	PD2	Low
ALL-4	ALL B-precursor	6.0	0.647	1.0	>25	.	PD1	Low
ALL-7	ALL B-precursor	6.0	0.647	1.0	>25	.	PD1	Low
ALL-8	ALL T-cell	>EP	<0.001	>7.5	14.0	.	CR	Int
ALL-17	ALL B-precursor	29.8	0.001	4.0	>25	.	PD2	Int
ALL-19	ALL B-precursor	>EP	0.001	>6.0	0.2	.	MCR	High
ALL-31	T-cell ALL	14.5	0.006	1.8	>25	.	PD2	Low
MLL-7	ALL B-precursor	12.5	0.077	2.1	>25	.	PD1	Low

¹ *Tumor Volume T/C value*: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C > 0.15; Intermediate activity = T/C > 0.15 but < 0.45; and Low activity = T/C > 0.45.

² Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS T/C > 1.5, and PD2 = progressive disease with EFS T/C > 1.5.

³ EFS T/C values = the ratio of the median time to event of the treatment group and the median time to event of the respective control group. High activity requires: a) an EFS T/C > 2; b) a significant difference in EFS distributions, and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Intermediate activity = criteria a) and b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity = EFS T/C < 2.