#1616 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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SELINEXOR

- Selinexor is an orally bioavailable Selective Inhibitor of Nuclear Export (SINE).
- Selinexor binds covalently to the nuclear export protein XPO1 at Cys528 resulting in irreversible inactivation.
- XPO1 exports over 200 proteins with specific nuclear export sequences.
- Cargo proteins include tumor suppressor proteins such as FOXO, IκB, pRB, p53, p73, p21 and p27.
- Selinexor is in clinical trials for adults with cancer.

PROCEDURES

- Selinexor was tested against the PPTP in vivo xenograft panels administered orally at a dose of 10 mg/kg (3 times per week) for 4 weeks.
- For collection of specimens for pharmacodynamic testing, KT-10 tumors that regressed 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.
- Immunoblots were probed for p53, p21, PARP and cleaved PARP and XPO1/CRM1.
- IHC analysis was performed for a comparable set of proteins to assess nuclear localization.
- For exome sequencing, all mutations were verified and assessed as somatic using a virtual normal subtraction algorithm

SELINEXOR IN VIVO ACTIVITY

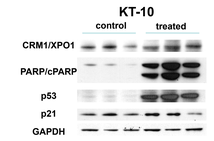
- Selinexor induced significant differences in event-free survival (EFS) distribution in 29 of 38 (76%) of the evaluable solid tumor xenografts and in 5 of 8 (63%) of the evaluable ALL xenografts.
- Objective responses (partial or complete responses, PR/CR) were observed for 4 of 38 solid tumor xenografts including Wilms tumor, medulloblastoma (n=2) and ependymoma models.
- For the ALL panel, 2 of 8 (25%) xenografts achieved either CR or maintained CR.

SELINEXOR *IN VIVO* ACTIVITY

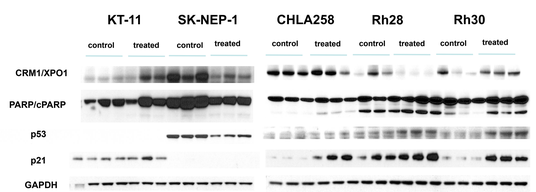
Line	Tumor Type	P-value	EFS T/C	Median RTV/CD45 at End of Study	Median Group Response
BT-29	Rhabdoid	<0.001	> 1.8	1.5	PD2
KT-16	Rhabdoid	<0.001	4.1	>4	PD2
KT-14	Rhabdoid	<0.001	> 1.7	2.3	PD2
KT-10	Wilms	<0.001	> 4.0	0.0	MCR
KT-11	Wilms	0.072	1.3	>4	PD1
KT-13	Wilms	<0.001	2.0	>4	PD2
SK-NEP-1	Ewing	<0.001	3.9	>4	PD2
EW5	Ewing	0.083	1.1	>4	PD1
EW8	Ewing	<0.001	2.7	>4	PD2
TC-71	Ewing	0.001	3.1	>4	PD2
CHLA258	Ewing	<0.001	3.3	>4	PD2
Rh10	Alveolar RMS	0.004	1.4	>4	PD1
Rh28	Alveolar RMS	0.006	1.5	>4	PD2
Rh30	Alveolar RMS	<0.001	3.2	>4	PD2
Rh30R	Alveolar RMS	<0.001	1.7	>4	PD2
Rh41	Alveolar RMS	<0.001	1.9	>4	PD2
Rh18	Embryonal RMS	<0.001	1.6	>4	PD2
BT-28	Medulloblastoma	<0.001	3.1	>4	PD2
BT-45	Medulloblastoma	0.47	NE	0.6	PR
BT-50	Medulloblastoma	<0.001	> 1.2	0.5	CR
BT-36	Ependymoma	<0.001	> 1.1	1.6	PD2
BT-41	Ependymoma	1.000	.	0.7	CR
GBM2	Glioblastoma	0.020	2.2	>4	PD2
BT-39	Glioblastoma	0.165	2.0	>4	PD2
D645	Glioblastoma	0.003	2.0	>4	PD2
D456	Glioblastoma	0.288	1.5	>4	PD1
NB-SD	Neuroblastoma	0.300	1.1	>4	PD1
NB-1771	Neuroblastoma	<0.001	2.0	>4	PD2
NB-1691	Neuroblastoma	0.165	1.2	>4	PD1
NB-EBc1	Neuroblastoma	0.046	1.4	>4	PD1
CHLA-79	Neuroblastoma	<0.001	4.3	>4	PD2
NB-1643	Neuroblastoma	0.010	1.2	>4	PD1
OS-1	Osteosarcoma	<0.001	1.3	>4	PD1
OS-2	Osteosarcoma	<0.001	1.1	>4	PD1
OS-17	Osteosarcoma	0.003	1.4	>4	PD1
OS-9	Osteosarcoma	<0.001	1.3	>4	PD1
OS-33	Osteosarcoma	<0.001	1.4	>4	PD1
OS-31	Osteosarcoma	0.315	1.0	>4	PD1
ALL-2	ALL B-precursor	0.003	1.9	>25	PD2
ALL-4	ALL B-precursor	0.647	1.0	>25	PD1
ALL-7	ALL B-precursor	0.647	1.0	>25	PD1
ALL-8	ALL T-cell	<0.001	> 7.5	14.0	CR
ALL-17	ALL B-precursor	0.001	4.0	>25	PD2
ALL-19	ALL B-precursor	0.001	> 6.0	0.2	MCR
ALL-31	T-cell ALL	0.006	1.8	>25	PD2
MLL-7	ALL B-precursor	0.077	2.1	>25	PD1

- **Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C between treated and control groups.**
- **Shading in the EFS and T/C columns indicates xenografts that have either high (dark blue) or intermediate (light blue) activity for these measures.**
- **PD1 (Progressive Disease 1, dark green):** >25% ↑ in tumor volume, TGD value ≤1.5
- **PD2 (Progressive Disease 2, light green):** >25% ↑ in tumor volume, TGD value >1.5
- **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression
- **PR (Partial response):** a tumor volume regression ≥50%
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** absence of measurable tumor mass at day 42.

SELINEXOR INDUCES TP53 PATHWAY GENES



Selinexor produces robust TP53 induction and PARP cleavage 24 hours after a single dose in the highly responsive KT-10 Wilms tumor xenograft.

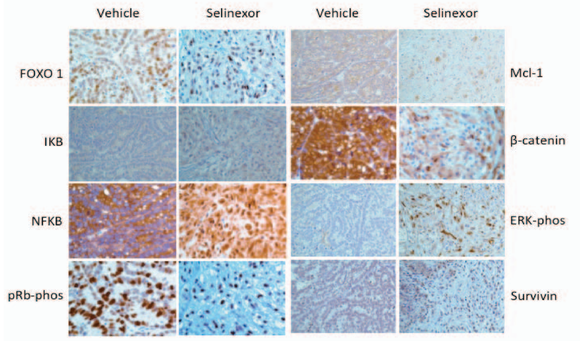


For three TP53 wildtype xenografts that showed significant tumor growth delay to selinexor (CHLA-258, Rh28, and Rh30), p21 induction was noted after 6 doses of selinexor. The TP53 mutated xenograft SK-NEP-1 showed noTP53 or p21 induction.

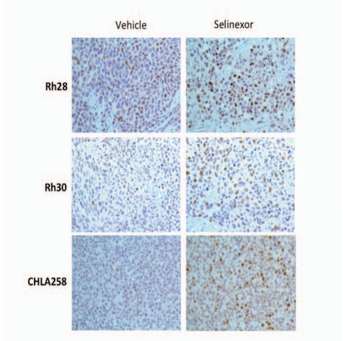
MUTATIONS IN RESPONDING XENOGRAFTS

- Gene mutation profiles were examined for five models with objective responses (KT-10, BT-45, BT-50, ALL-8, and ALL-19).
- Two genes were mutated in two models: *FBXW7* and *SMARCA4*.
- For *FBXW7*, known oncogenic mutations were identified for both BT-50 (R465H) and ALL-8 (R465C). BT-50 also had a *SUFU* mutation (D182G), consistent with SHH pathway medulloblastoma.
- 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 and BT-45 also showed objective responses to cisplatin. KT-10 has a *PALB2* mutation that leads to defective homologous recombination and to sensitivity to PARP inhibitors as well as to cisplatin. BT-45 has a mutation profile consistent with WNT pathway medulloblastoma (*CTNNB1* and *TP53* mutation).
- Further preclinical research and clinical experience will be required to determine if any of the mutated genes noted above are able to predict for response to selinexor.

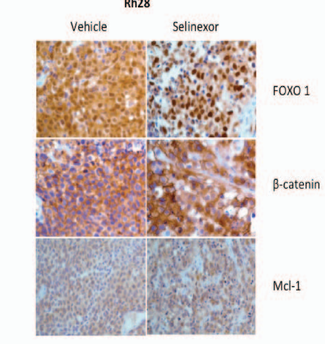
SELINEXOR INDUCES NUCLEAR ACCUMULATION OF XPO1 CARGO PROTEINS IN PEDIATRIC CANCER MODELS



Sections from vehicle and selinexor treated KT-10 were analyzed by immunohistochemistry (IHC). XPO1 cargo proteins were blocked in the nucleus and the expression of signaling proteins that are associated with cell proliferation was reduced. 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



In vivo treatment with selinexor induces nuclear p53 accumulation in tumors with tumor growth delay to selinexor. Sections from vehicle and selinexor treated Rh28, Rh30 and CHLA258 were analyzed by IHC.



For Rh28, which showed limited tumor growth inhibition to selinexor, there was only partial FOXO3 nuclear accumulation with much cytoplasmic stain. In addition, there was minimal reduction of β-catenin and almost no reduction in Mcl-1 protein.

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