PRECLINICAL ACTIVITY OF SELINEXOR, AN INHIBITOR OF XPO1, IN SARCOMA

Abstract #1759

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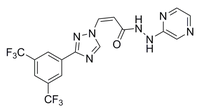
➢ Introduction



SELINEXOR(KPT-330)

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i. XPO1 exports nuclear cargo proteins, including tumor suppressors (e.g. p53), cell cycle regulators (e.g. p21), and many more.

ii. Selective Inhibitors of Nuclear Export (SINE) that block XPO1 from binding to cargo proteins are in clinical trials as anticancer therapeutics.

iii. We evaluated the effects of selinexor, an orally bioavailable SINE, in preclinical models of sarcoma.

➢ Conclusions

1. **Selinexor has potent *in vitro* and *in vivo* activity against a wide variety of sarcoma models.**
2. **Selinexor induced G_1-arrest independent of known molecular mechanisms in GIST and LPS.**
3. **These studies further justify the exploration of selinexor in clinical trials targeting various sarcoma subtypes.**

➢ Acknowledgement

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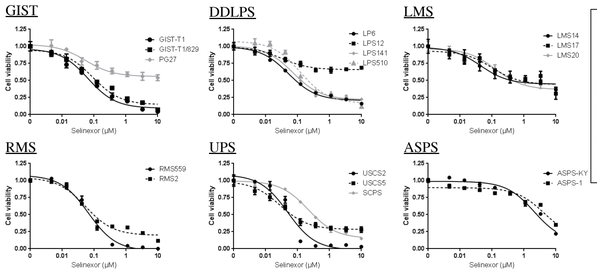
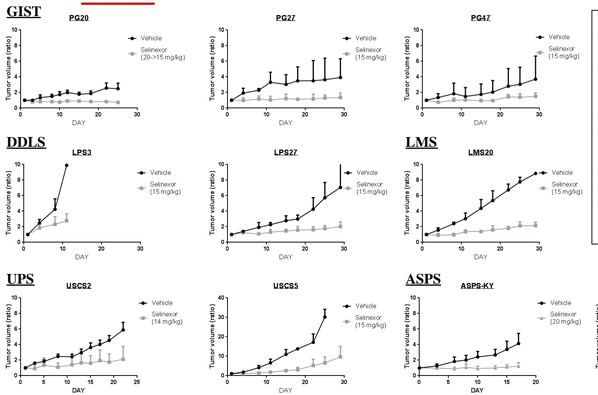
➢ Fig.1 Anti-proliferative activity of selinexor in a variety of sarcoma cell lines *in vitro*.



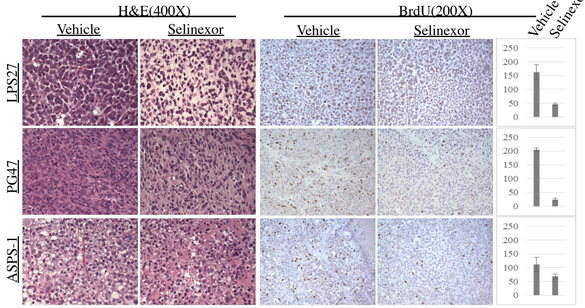
Cell viability was measured using Cell Titer Glo. Most sarcoma cell lines were sensitive to selinexor with IC_{50}s ranging from 28.8 nM to 218.2 nM (median: 66.1 nM).
The ASPS cell lines were exceptionally resistant to selinexor with IC50 greater than 2 μM.
These data demonstrate that many but not all sarcoma histologic subtypes are sensitive to selinexor *in vitro*.

➢ Fig.2 Anti-proliferative activity of selinexor in a variety of sarcoma models *in vivo*.



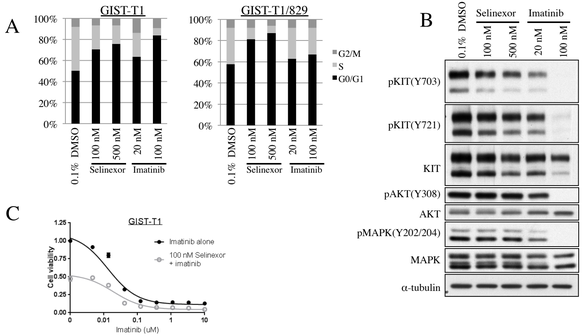
In sarcoma xenograft studies, either cryopreserved tumors or cell lines mixed 1:1 with Matrigel were subcutaneously implanted into the flanks of nude mice. Treatment began when tumors reached an average size of 100 mm^3. Selinexor was administered twice weekly by oral gavage. Three mice were treated either with control or selinexor in each cohort. The Y-axis indicates average changes in volume from day 1. Selinexor suppressed tumor growth, including ASPS models that were resistant *in vitro*.

➢ Fig.3 Histological changes and reduced cell proliferation following selinexor treatment.



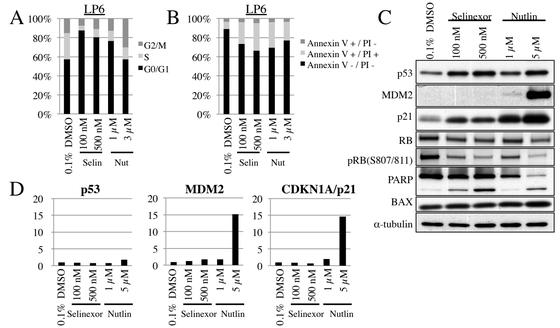
BrdU solution was injected intraperitoneally 22 hours after the last drug administration. After 2 additional hours, tumors were harvested and fixed for histologic analysis. BrdU positive cells were counted in three representative fields at 200x magnification and compared between two groups (right graphs).
LPS27: The tumor cells treated with selenexor showed smaller nuclei, some with a pyknotic appearance, and abundant clear cytoplasm, whereas the control tumors showed sheets of large round cells with vesicular chromatin, prominent nucleoli, frequent mitotic figures, and minimal cytoplasm.
PG47(GIST): The treated tumor did not show any appreciable difference in H&E.
ASPS-1: The treated tumor showed loss of delicate capillary vasculature and alveolar/ nested architecture.

➢ Fig.4 Selinexor induced cell cycle arrest in GIST independent of KIT signaling pathway.



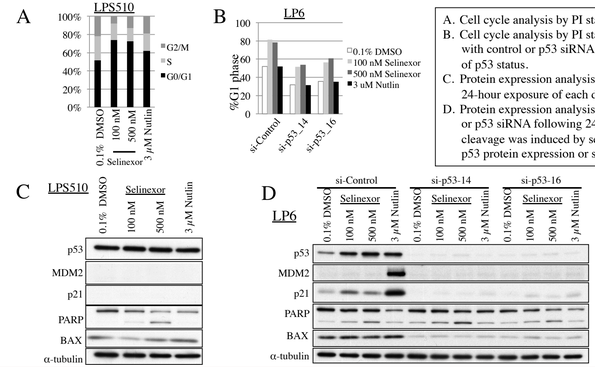
A. Cell cycle analysis by propidium iodide (PI) staining in the GIST-T1 line and the imatinib-resistant GIST-T1/829 subclone. Selinexor induced G_1-arrest in a dose-dependent manner irrespective of the presence of secondary *KIT* mutation.
B. Protein expression analysis in the GIST-T1 line following 24-hour exposure of each drug. Selinexor exhibited no effect on the phosphorylation of downstream molecules (AKT and MAPK), whereas imatinib caused a dramatic decrease in phosphorylation of KIT as well as of downstream molecules
C. Cell viability assay in the GIST-T1 line following the 72-hour exposure to the serial concentration of imatinib (IM) with or without 100 nM selinexor. The combination of selinexor and imatinib showed an additive effect in cell viability assays.

➢ Fig.5 Selinexor induced cell cycle arrest and apoptosis in LPS differently from Nutlin-3a.



A. Cell cycle analysis by PI staining in the LP6 line.
B. Apoptosis analysis by annexin V / PI staining in the LP6 line. Selinexor induced both G_1-arrest and apoptosis in LP6 cells at 100 nM.
C. Protein expression analysis in the LP6 line following 24-hour exposure of each drug.
D. Gene expression analysis of p53, MDM2 and CDKN1A /p21 in the LP6 by qPCR, showing no increase in p53, MDM2, or CDKN1A/p21 RNA level following treatment of LP6 with selinexor whereas significant induction of MDM2 and CDKN1A /p21 RNA expression followed treatment with Nutlin-3a.

➢ Fig.6 Selinexor acts independently of p53 in LPS.



A. Cell cycle analysis by PI staining in the p53-mutant LPS510 line.
B. Cell cycle analysis by PI staining in the LP6 lines that were treated with control or p53 siRNA. Selinexor induced G_1-arrest irrespective of p53 status.
C. Protein expression analysis in the p53-mutant LPS510 line following 24-hour exposure of each drug.
D. Protein expression analysis in the LP6 lines transfected with control or p53 siRNA following 24-hour exposure of each drug. PARP cleavage was induced by selinexor, without significant increase in p53 protein expression or significant expression of p21 and MDM2.