KPT-8602 is a second-generation XPO1 inhibitor with improved *in vivo* tolerability that demonstrates potent acute lymphoblastic leukemia activity

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Background

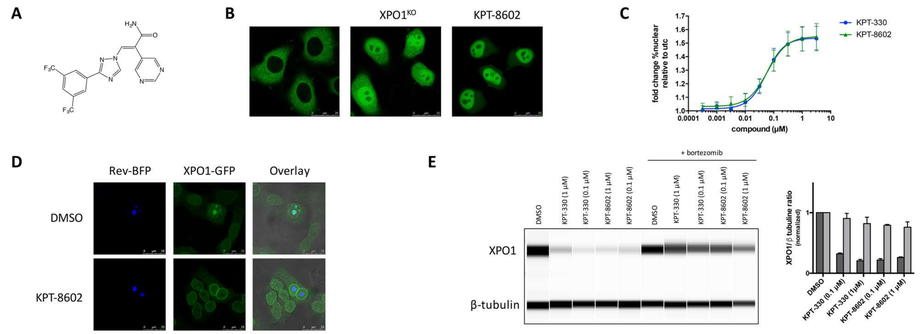
Human exportin-1 (XPO1), or chromosome region maintenance 1 protein (CRM1), is a key nuclear-cytoplasmic transport protein. It exports a broad range of different cargo proteins out of the cell's nucleus to the cytoplasm. These cargo proteins include tumor suppressor and growth regulatory related proteins; therefore correct XPO1 function is key to normal cell homeostasis. In recent years, overexpression or dysfunction of XPO1 has commonly been observed in different types of cancer and alterations in XPO1 expression levels may cause subcellular mislocalization of tumor suppressor proteins and cell cycle regulators, resulting in uncontrolled cell growth and carcinogenesis. Therefore, XPO1 is considered a potential anti-cancer target. First clinical validation of targeting XPO1 function by small molecules was obtained with the oral Selective Inhibitor of Nuclear Export (SINE) compound selinexor (KPT-330) that is currently in Phase-II/IIb clinical trials. However, tolerability in humans only allows dosing of selinexor every other day 1 - 3 times a week. Therefore XPO1 inhibitors with improved tolerability allowing more frequent dosing may have substantial clinical benefit. Here we present a second generation SINE compound KPT-8602 with improved tolerability that can be dosed daily. We demonstrate potent inhibitory activity against various leukemia cell lines as well as strong *in vivo* activity for the treatment of acute lymphoblastic leukemia (ALL). ALL is a severe hematologic cancer with a peak incidence in young children. Although the overall survival is now >90% for childhood ALL, the outcome in infants, adults and relapse patients remains poor. In addition, treatment of ALL consists of toxic chemotherapeutic dosing schemes with severe long-term side effects, including the risk of developing a secondary malignancy. Newly discovered targeted therapies usually inhibit one specific genomic aberration, limiting treatment to only a small subset of patients. Compounds that target nuclear export, such as selinexor or KPT-8602, could potentially be used for the treatment of different ALL subtypes, independent of molecular characteristics.

Conclusion

Our data demonstrate that the second generation SINE compound KPT-8602 is a potent XPO1 inhibitor, directly interacting with XPO1, that can effectively inhibit leukemia growth *in vivo* with minimal effect on normal hematopoiesis.

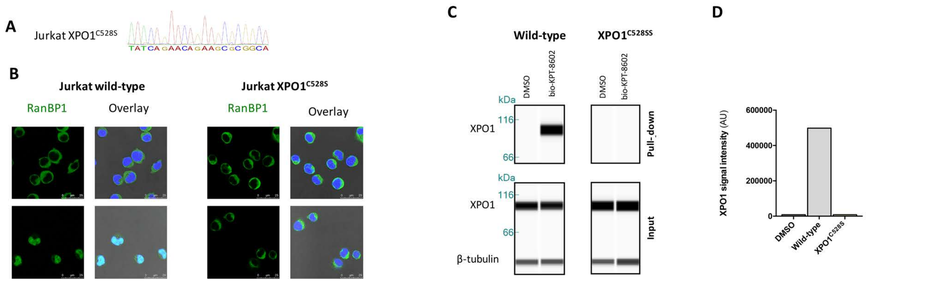
KPT-8602 inhibits XPO1-mediated cargo export

To assess whether KPT-8602 (A) is able to inhibit XPO1-mediated nuclear export of cargo protein, it was tested in a phenotypic reporter assay (B). This assay uses a GFP reporter protein that is fused to a NES and a NLS, which cause the GFP reporter to actively shuttle between nucleus and cytoplasm. In steady state the GFP fusion is mainly localized to the cytoplasm in a XPO1-dependent manner as demonstrated by CRISPR/Cas9 knock out of XPO1 as this resulted in accumulation of the GFP reporter in the nucleus. HeLa cells stably expressing this GFP reporter protein were treated with increasing concentrations of KPT-8602 and the localization of GFP was determined by imaging (C). KPT-8602 inhibited the XPO1 dependent nuclear export with an EC_{50} value of 60.9 ± 3.6 nM, which is similar to the EC_{50} values for the first-generation XPO1 inhibitor selinexor (55.7 ± 6.5 nM). Co-localization experiments further demonstrate that KPT-8602 is blocking the interaction of XPO1 with Rev cargo (D). In addition to the direct inhibition of XPO1-cargo binding, KPT-8062 also caused proteolytic degradation of XPO1 protein (E).



KPT-8602 interacts with XPO1

To demonstrate that KPT-8602 is inhibiting the XPO1-mediated nuclear export by directly targeting XPO1 we applied CRISPR/Cas9 genome editing to introduce a resistance Cys528Ser mutation in the *XPO1* gene of Jurkat leukemia cells (A). The effect of the mutation on KPT-8602 activity was assessed by the visualization of the subcellular localization of the RanBP1 cargo (B). To further demonstrate the direct binding of KPT-8602 to XPO1 in cells, Jurkat cells were treated with biotinylated KPT-8602 (KPT-9511), lysed and streptavidin affinity purified. The eluate was analyzed for co-precipitated XPO1 protein using simple western immunoblotting (C, D).

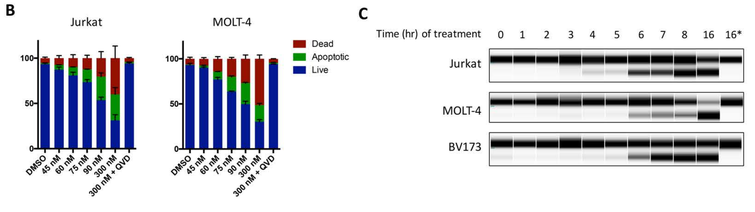


KPT-8602 induces apoptosis in leukemia cell lines

The anti-leukemic activity of KPT-8602 was assessed on several varieties of leukemia cell lines (T-ALL, B-ALL and AML) (A). Cell viability was reduced in all KPT-8602-treated leukemia cell lines with EC_{50} values ranging from 25 to 145 nM. Decrease in leukemia cell viability was further confirmed by annexin V/PI staining of Jurkat and MOLT-4 T-ALL cells. Induction of apoptosis was also confirmed by the appearance of cleaved caspase-3 substrate PARP as early as 6 hours after treatment with 1 µM KPT-8602 (C).

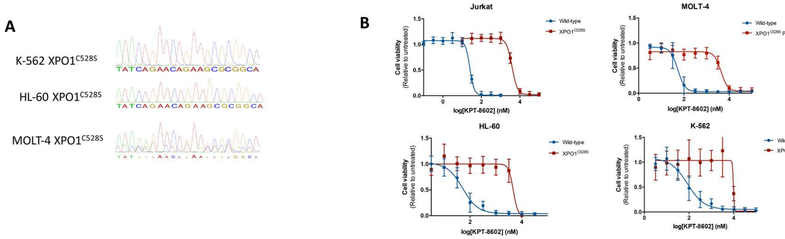
A

Cell line	EC_{50} (nM)
MV4-11	36 ± 3
MOLM13	25 ± 6
HL-60	58 ± 33
K562	86 ± 73
BV173	55 ± 15
EHEB	131 ± 40
REH	79 ± 29
MOLT-4	63 ± 26
Jurkat	26 ± 3
DND41	52 ± 5
HPB-ALL	143 ± 28
ALL-SIL	91 ± 32



Drug target validation

To prove that the observed anti-leukemic activity of KPT-8602 was directly caused by inhibition of XPO1 and not by off-target effects we tested the activity of KPT-8602 on 4 leukemia cell lines (Jurkat, MOLT-4, HL-60 and K562) in which we introduced the $XPO1^{C528S}$ mutation by CRISPR/Cas9 genome editing (A). Mutant leukemia cells containing the single $XPO1^{C528S}$ mutation were 60-150 times more resistant to KPT-8602 than the parental cells (B) demonstrating that the C528S mutation confers resistance to the drug. These results demonstrate that XPO1 is the target for KPT-8602 and that its anti-leukemic activity is caused by inhibition of XPO1-mediated nuclear protein export.



KPT-8602 prolongs xenograft survival

To determine the activity of KPT-8602 against human leukemic cells *in vivo* we used a patient derived xenograft (PDX) model. First, treatment was initiated at day 10 after injection, when human leukemia cells in the PB of the mice ranged from 0 - 0.5%. Mice that received KPT-8602 had significantly lower numbers of human leukemia cells in their PB as compared to placebo (A). Next, treatment was started when mice had minimum 5% human leukemia cells in their PB and survival was of 5 KPT-8602 and 5 placebo treated PDX-mice was analyzed (B)