THE COMBINATION OF SELINEXOR (KPT-330), A SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE), & THE FLT3 INHIBITOR QUIZARTINIB SHOWS ANTI-TUMOR ACTIVITY IN ACUTE MYELOID LEUKEMIA (AML) IN-VITRO AND IN-VIVO

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Abstract P796

Abstract

Background

AML cells overexpress the nuclear exporter Exportin 1 (XPO1/CRM1) and higher XPO1 levels correlate with poor outcomes in AML and other cancers. Selinexor (KPT-330), a novel SINE, antagonizes XPO1 and shows potent cytotoxicity for AML cells *in-vitro* and *in-vivo*, independent of genotype, while largely sparing normal hematopoietic cells. Mechanistic studies show that selinexor induces nuclear localization and activation of multiple tumor suppressor proteins (TSPs) and reduces levels of oncoproteins such as Flt3 and KIT, leading to rapid apoptosis of AML cells. We have recently reported preliminary Phase 1 results in which treatment with oral selinexor has led to durable disease stabilization and responses in patients with relapsed/refractory AML across multiple genotypes. Quizartinib is a selective inhibitor of Flt3, a receptor tyrosine kinase for which the FLT3-ITD mutant is frequently a driver for AML. Quizartinib has shown significant activity in AML and the drug is scheduled to enter Phase 3 in FLT3-ITD+ AML patients this year. We report here the results of *in-vitro* and *in-vivo* studies of Selinexor and quizartinib alone or in combination on proliferation and survival of MV4-11 AML cells.

Aims

To characterize the *in-vitro* and *in-vivo* effects of combining selinexor with quizartinib on AML cell survival relative to treatment with either drug alone.

Methods

FLT3-ITD+ MV-4-11 AML cells were used for cell culture and xenograft studies. CellTiter-Glo® was used to measure *in-vitro* cell proliferation. For xenograft studies, MV4-11 cells were grown as subcutaneous tumors in NOD-SCID mice.

Results

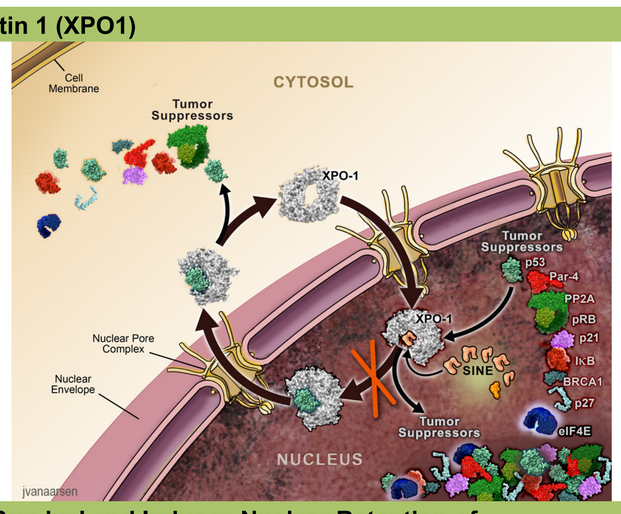
Selinexor and quizartinib each showed highly potent inhibition of MV-4-11 proliferation *in-vitro*, and the combination showed synergistic potency. In the MV-4-11 AML xenograft model in NOD-SCID mice, a combination of selinexor with quizartinib also had an apparently synergistic effect on tumor growth relative to the drugs administered as monotherapies. Suboptimal doses of selinexor (5 or 10 mg/kg PO QODX3) had no effect or induced a 60% median tumor growth inhibition (TGI), respectively, by the end of the study. Quizartinib at 0.5 or 10 mg/kg QDX7 PO induced 60% TGI or 91% regression, respectively, over the same timeframe. The combination of the suboptimal doses of selinexor (5 mg/kg) and quizartinib (0.5 mg/kg) induced 67% tumor regression. While 10 mg/kg quizartinib induced modest weight loss, selinexor, 0.5 mg/kg quizartinib, and the combination were well-tolerated with expected weight gain and full survival.

Summary/Conclusions

Selinexor is a potent inhibitor of *in-vitro* and *in-vivo* AML cell survival and functions by nuclear localization / activation of TSPs and reduction in FLT3 and other oncoproteins. Direct Flt3 inhibition with quizartinib also results in killing of Flt3 abnormal AML cells. The combination of selinexor and quizartinib shows additive cytotoxicity on AML cells *in-vitro*. Moreover, the combination of suboptimal doses of selinexor with quizartinib *in-vivo* was well tolerated and was dramatically more effective than either drug alone, leading to robust xenograft regression. This synergistic effect provides rationale for the investigation of a selinexor/quizartinib combination in the clinic with the potential for enhanced efficacy in AML relative to treatment with these drugs as monotherapies.

Exportin 1 (XPO1)

- Exportin-1 (XPO1; Crm1) is the major nuclear exporter with >200 protein cargos, including proteins and some RNAs central to carcinogenesis, viral replication, and inflammation.
- XPO1 is overexpressed in AML and its levels correlate with poor prognosis.
- SINEs induce nuclear retention of proteins and RNAs to exert effects beneficial for a variety of disease states, including cancer, viral infection, brain damage and a variety of autoinflammatory conditions.
- Selinexor, the most advanced SINE, has been tested in >300 patients to date in three ongoing Phase 1 trial, with promising signs of efficacy, tolerability and safety.



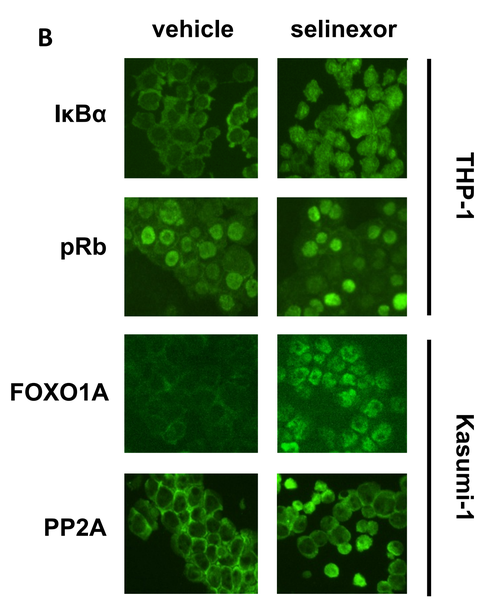
Selinexor Potently Inhibits In-Vitro Survival and Induces Nuclear Retention of XPO1 Cargo Proteins in AML Cell Lines

A

AML Cell Line	IC_{50} (μM)
EOL1	0.009
SIG-M5	0.016
MOLM-13*	0.022
MV-4-11*	0.022
CMK	0.026
TF-1	0.060
M-07e	0.065
OCI-M1	0.075
OCI-AML2	0.080
OCI-AML5	0.12
PL-21*	0.16
GDM-1	0.16
MOLM-16	0.21
MUTZ-3	0.21
KG-1	0.24
Hel	0.35
F-36P	0.38
SKM-1	0.38
Hel 92.1.7	0.44
Kasumi-1	0.54
AML-193	0.54
THP-1	0.80
OCI-AML3	2.5
Kasumi-6	>10

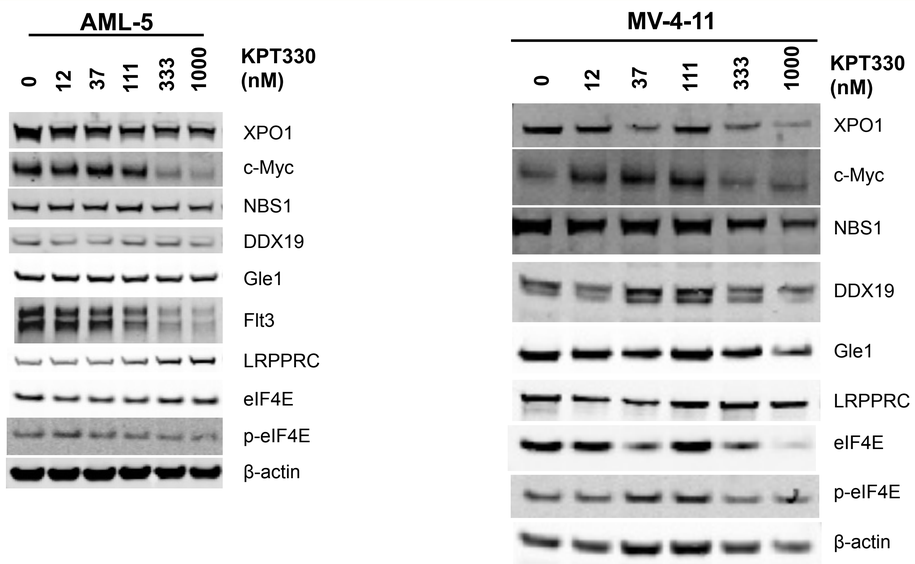
*FLT3-ITD+

B



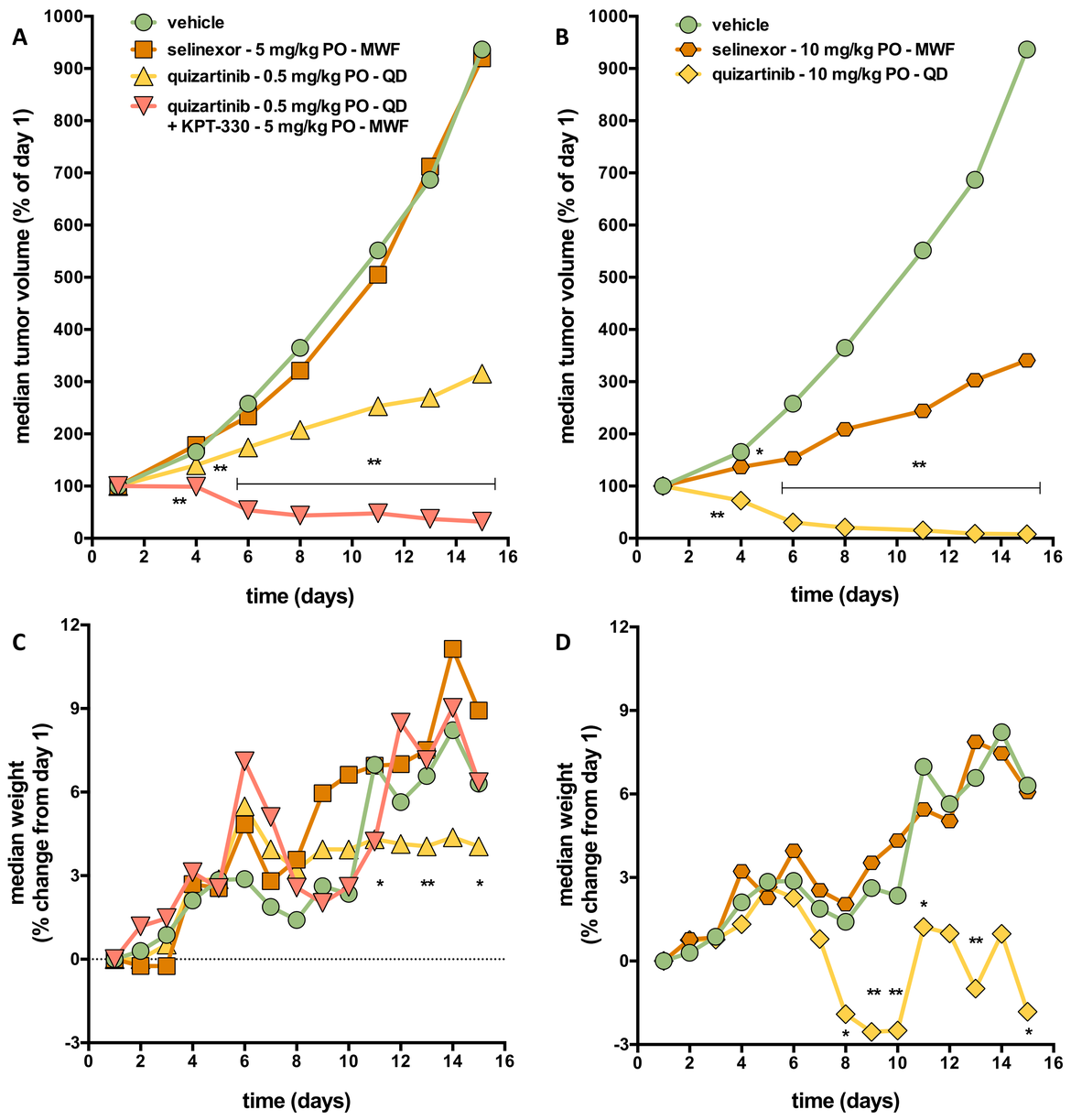
(A) Selinexor in vitro potency for inhibition of survival was determined across a panel of 24 AML cell lines. Cells were incubated with a range of selinexor concentrations for 72 h and surviving cells were quantified using CellTiter-Glo®. Median selinexor potency across the panel was 162 nM. **(B)** Fluorescent immunohistochemistry of the XPO1 protein cargos IKBα, pRb, FOXO1A and PP2A after treatment of THP-1 or Kasumi-1 cells with 1 μM selinexor for 8 h. Protein levels of all four cargos were significantly increased in the nucleus following selinexor treatment.

Selinexor Affects eIF4e-Related AML Cell Biology



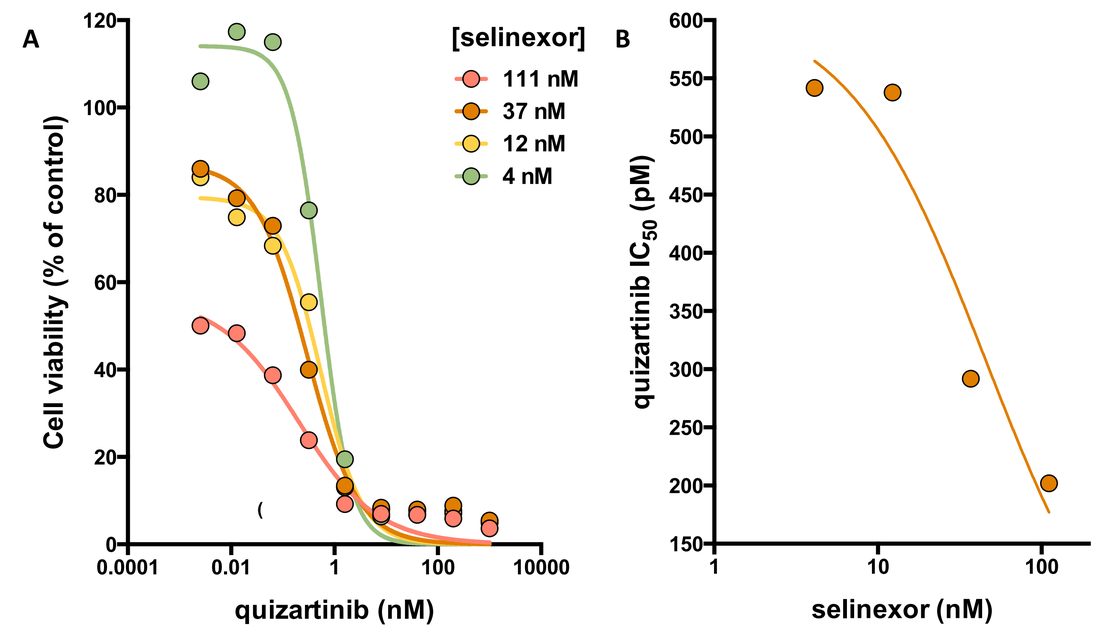
The oncogene eIF4E is frequently overexpressed in AML and eIF4E mRNA and associated capped mRNAs are dependent upon XPO1 for nuclear export (Culjkovic-Kraljacic et al. (2012) Cell Reports 2, 207-15). Nuclear retention of capped mRNAs associated with eIF4E mRNA through XPO1 should therefore result in decreased expression of proteins translated from those capped RNAs. Protein levels of XPO1, eIF4E and a variety of proteins that arise from capped mRNAs (c-Myc, NBS1, DDX19, Gle1, Flt3) were measured by Western Blot in the OCI-AML5 and MV-4-11 AML cell lines after 24 h treatment with a range of selinexor doses. The most dramatic reductions in capped mRNA proteins was observed for c-Myc and Flt3, both of which are important AML oncogenes. (Flt3 was not detectable in MV-4-11.)

Combination Therapy with Selinexor and Quizartinib Synergistically Induces Tumor Regression in an AML Xenograft Model



NOD-SCID mice were inoculated in the left flank with $4x10^6$ MV-4-11 cells suspended in Matrigel (BD Biosciences CB-40234). When resulting solid tumors reached ~100 mm^3, mice were randomized into cohorts of eight for treatment with vehicle, selinexor (5 or 10 mg/kg QODx3/wk PO), quizartinib (0.5 or 10 mg/kg QD PO) or a combination of selinexor (5 mg/kg) and quizartinib (0.5 mg/kg) over the two week experiment. The mice were weighed daily and tumor volume was measured using calipers on days 1, 4, 6, 8, 11, 13 and 15. Mann Whitney test was used to determine statistical significance, as indicated on the graphs (*p<0.05; **p<0.01).

Selinexor Acts Synergistically with Quizartinib to Reduce AML Cell Line Survival



(A) The effect of combining selinexor with quizartinib on in vitro survival of the MV-4-11 cell line was evaluated. The graph depicts the dose response of quizartinib in the presence of a range of selinexor concentrations. IC_{50} values for selinexor or quizartinib alone were 81 nM and 450 pM, respectively. **B)** Relative quizartinib IC_{50} values determined in (B) as a function of selinexor concentration, demonstrating that increasing dose of selinexor synergistically increases the potency of quizartinib.

Results Summary and Conclusions

Results

- The XPO1 inhibitor selinexor potently inhibited in vitro survival across a large panel of AML cell lines and induces nuclear retention of XPO1 cargo proteins cargos IKBα, pRb, FOXO1A and PP2A.
- The combination of selinexor with Flt3 inhibitor quizartinib showed synergistic potency against in vitro survival of the AML cell line MV-4-11.
- Selinexor induces decreased levels of proteins derived from capped mRNAs associated with eIF4E mRNA (including Flt3), which are dependent upon XPO1 for nuclear export.
- In MV-4-11 xenografts, an ineffective dose of selinexor combined synergistically with a partially effective dose of quizartinib to induce tumor regression.

Conclusions

- Selinexor-induced decrease in Flt3 protein level may at least in part be the basis for synergistic cytotoxicity with quizartinib in AML cells and xenografts.
- Based upon in vitro and in vivo synergy of a combination of selinexor and quizartinib and the apparent efficacy and safety of selinexor as monotherapy in AML patients to date, the combination of selinexor with quizartinib or other Flt3 inhibitors may prove effective for treating AML.

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