DANA-FARBER Boston Children's
CANCER AND BLOOD DISORDERS CENTER

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

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Selective Inhibitor of Nuclear Exporter CRM1/XPO1, Selinexor (KPT-330), Exhibits Remarkable Activity against AML Leukemia-initiating Cells while Sparing Normal Hematopoietic cells

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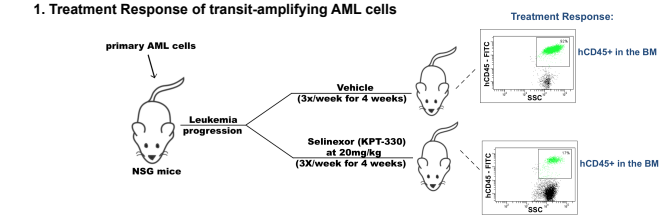
Karyopharm Therapeutics

Current treatments for acute myeloid leukemia (AML) often fail to induce long-term remissions and are toxic to normal tissues, prompting the need to develop new targeted therapies. The frequent disease relapse that is observed in patients with AML is thought to occur because of the inability of the existing drugs to target the self-renewing leukemia-initiating cells (LICs). An attractive new strategy for AML therapy is inhibition of the nuclear export protein exporter 1 (XPO1), or CRM1. XPO1 regulates export of proteins that contain leucine-rich nuclear export signals (NES), including protein adaptors that mediate transport of RNA. XPO1 cargo encompass tumor suppressor proteins, cell cycle regulators, and apoptotic proteins. Recently, small molecule inhibitors of nuclear export (SINE) that inhibit the export function of XPO1 by targeting Cys^{528} in its NES-binding groove, were developed using an *in silico* molecular modeling. Selinexor (KPT-330), the orally bioavailable SINE compound, is in Phase 1 and 2 studies in adult patients with AML (NCT01607892 and NCT02088541). In 2014, selinexor entered phase I trial in children with relapsed or refractory AML or ALL (NCT02091245), and phase I and II trials to evaluate its activity in combination with chemotherapeutic drugs in patients with relapsed or refractory AML (NCT02249091, NCT02212561, NCT02088541, NCT02093403, NCT02299518).

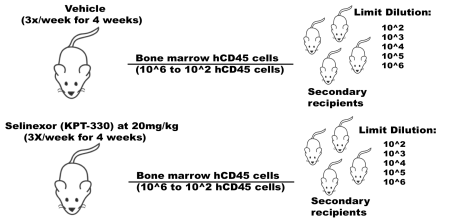
To define the anti-leukemic activity of selinexor against primary AML blasts and LICs in a clinically relevant setting, we established mouse models of primary human leukemia, or patient-derived xenografts (PDX), in which leukemic blasts from AML patients were transplanted into immunodeficient NOD-SCID-IL2Rcγ^{null} (NSG) mice. Mice engrafted with leukemic blasts were treated with either vehicle or selinexor. Selinexor was highly active against blast cells from two of the three patients with poor-prognosis disease (cytogenetically normal AML with FLT3-ITD (AML-CN) and complex karyotype AML (AML-CK1 and AML-CK2)), as evidenced by a reduction in leukemic engraftment in primary mice after 4 weeks of treatment. Secondary transplantation assays indicated that selinexor greatly reduced the frequency of LICs in PDX models derived from all three patients (6- to 430- fold reduction compared to controls), indicating that this agent not only targets the bulk leukemic cells, but also eliminates LICs. These findings show that selinexor has potent activity against LICs, even when it has only moderate activity against the bulk AML cell population. Furthermore, preliminary results of combination studies of selinexor with Ara-C, a standard chemotherapeutic agent, demonstrate synergistic effect of the two drugs against LICs in a PDX model of AML-CN. Importantly, the fold decrease in absolute counts of normal human hematopoietic stem and progenitor cells in human CD34+ grafts after 4 weeks of selinexor therapy was much lower that the fold reduction in LIC frequency of the AML cells isolated from selinexor –treated xenografts, indicating that selinexor provides a therapeutic window for targeting LICs. These findings demonstrate that inhibition of nuclear export with selinexor overcomes an important obstacle to cure of AML, which is to destroy the very critical LIC compartment while sparing normal hematopoietic cells.

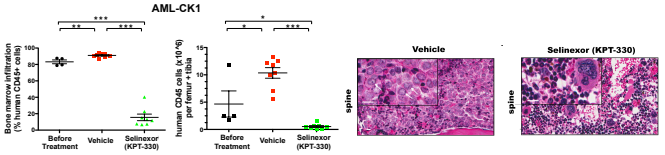
Selinexor (KPT-330) suppresses the growth of primary AML cells, including AML Leukemia Initiating Cells (LICs)

1. Treatment Response of transit-amplifying AML cells

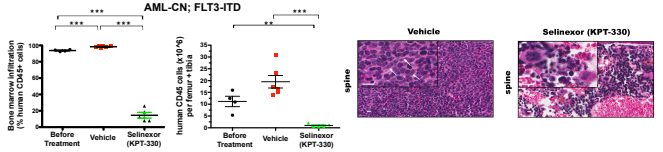


2. Treatment Response of Leukemia-Initiating AML cells

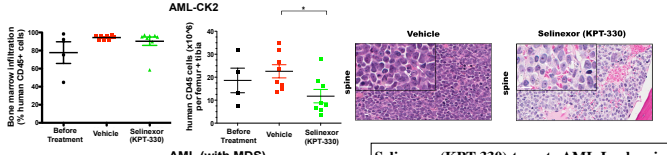




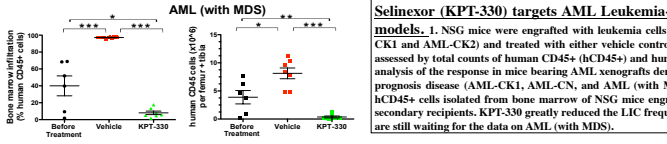
Xenograft AML-CK1	5x10^5 (Leukemic/ Total)	10^5 (Leukemic/ Total)	10^4 (Leukemic/ Total)	10^3 (Leukemic/ Total)	10^2 (Leukemic/ Total)	LIC Freq. (p < 0.05)	95% Conf. (Lower-Upper)	Reduction in LIC frequency (KPT/Veh)
Vehicle	6/6	6/6	5/5	3/6	0/6	~1/1615	(4648-561)	5.96 fold
KPT-330	6/6	6/6	3/5	1/6	0/6	~1/9631	(26950-3442)	



Xenograft AML-CN	1.25x10^5 (Leukemic/ Total)	6.25x10^4 (Leukemic/ Total)	2.5x10^4 (Leukemic/ Total)	10^3 (Leukemic/ Total)	5x10^2 (Leukemic/ Total)	10^2 (Leukemic/ Total)	LIC Freq. (p<0.0001)	95% Conf. (Lower-Upper)	Reduction in LIC frequency (KPT/Veh)
Vehicle				2/7	1/8	0/8	~1/3499	(10814 -1133)	171 fold
KPT-330	2/5	0/8	0/8				~1/597842	(2341609 -152637)	

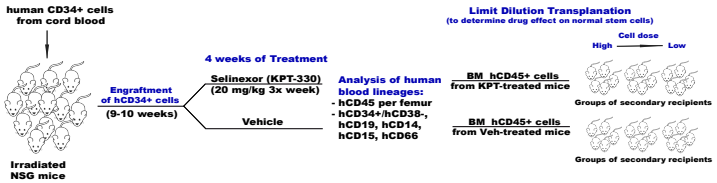


Xenograft AML-CK2	10^6 (Leukemic/ Total)	10^5 (Leukemic/ Total)	10^4 (Leukemic/ Total)	10^3 (Leukemic/ Total)	10^2 (Leukemic/ Total)	LIC Freq.	95% Conf. (Lower-Upper)	Reduction in LIC frequency (KPT/Veh)
Vehicle	6/6	6/6	6/6	6/6	6/6	>1/100		>434 fold
KPT-330	6/6	6/6	6/6	0/6	0/6	~1/43413	(105107-17931)	

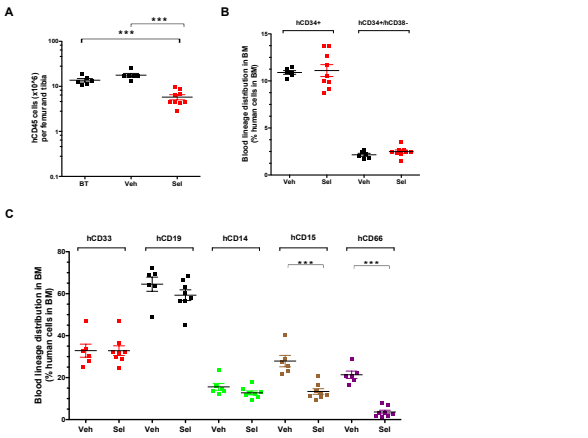


Selinexor (KPT-330) targets AML Leukemia-Initiating Cells (LICs) in mouse primary patient-derived xenograft (PDX) models. 1. NSG mice were engrafted with leukemia cells isolated from patients with M4-AML; FLT3-ITD (AML-CN) or complex karyotype disease (AML-CK1 and AML-CK2) and treated with either vehicle control or KPT-330 at 20 mg/kg for 4 weeks. After treatment, the extent of leukemic cell infiltration was assessed by total counts of human CD45+ (hCD45+) and human CD33+ (hCD33+) cells per mouse femur and tibia in vehicle- and KPT-330- treated animals. The analysis of the response in mice bearing AML xenografts demonstrates that selinexor is highly active against blast cells from three of the four patients with poor-prognosis disease (AML-CK1, AML-CN, and AML (with MDS)). 2. To determine LIC frequency for vehicle and KPT-330 treated mice, serial dilutions of hCD45+ cells isolated from bone marrow of NSG mice engrafted with primary leukemia and treated with either vehicle or KPT-330 were re-transplanted into secondary recipients. KPT-330 greatly reduced the LIC frequency of AML-CN and two complex karyotype AML patient samples (AML-CK1 and AML-CK2). We are still waiting for the data on AML (with MDS).

Selinexor (KPT-330) has low toxicity against normal human CD34+ engrafted into NSG mice



Analysis of Human Hematopoietic Lineages after 4 weeks of Selinexor Therapy



Selinexor (KPT-330) has little cytotoxicity against normal human hematopoietic cells engrafted into NSG mice. (A) Total counts of hCD45+ cells per femur and tibia in mice engrafted with cord blood hCD34+ cells before and after treatment with either vehicle or selinexor at 20 mg/kg for 4 weeks. (B) Percentage of hCD34+ and hCD34+/CD38- cells in the BM of established grafts treated with either vehicle or selinexor. (C) Distribution of human blood lineages in the bone marrow of grafts treated with either vehicle or selinexor. Selinexor (Sel), Before Treatment (BT). n=6-9 mice per group; Error bars represent mean +/- SEM; ***p < 0.0001 by Student's t-test for comparison between the indicated groups