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ORIGINAL REPORT

Phase I Study of Selinexor, a Selective Inhibitor of Nuclear Export, in Combination With Fludarabine and Cytarabine, in Pediatric Relapsed or Refractory Acute Leukemia

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ABSTRACT

Purpose

To characterize the toxicity, pharmacokinetics, and pharmacodynamics of selinexor, a selective inhibitor of nuclear export, when combined with fludarabine and cytarabine, in children with relapsed or refractory leukemia.

Patients and Methods

Eighteen patients with relapsed or refractory acute leukemia were enrolled in the SELHEM (Selinexor With Fludarabine and Cytarabine for Treatment of Refractory or Relapsed Leukemia or Myelodysplastic Syndrome) clinical trial (NCT02212561). Selinexor, initially at 30 mg/m^2 per dose, was given orally on days 1, 3, 8, 10, 22, and 24 and was escalated according to a rolling-six design. Fludarabine 30 mg/m^2 and cytarabine 2 g/m^2 were administered on days 15 to 19. Pharmacokinetic and pharmacodynamic studies were performed on days 1 and 22. Response evaluations were performed on day 15 and at the completion of course 1.

Results

Among the 17 patients who were evaluable for toxicity, three were treated at 30 mg/m^2, three at 40 mg/m^2, six at 55 mg/m^2, and five at 70 mg/m^2. The most common grade 3 nonhematologic toxicity was asymptomatic hyponatremia. Two patients who were treated at 70 mg/m^2 experienced reversible cerebellar toxicity, thereby defining the dose-limiting toxicity. Pharmacokinetic parameters demonstrated that plasma exposure was dose proportional. Fifteen of 16 patients demonstrated at least a twofold increase of *XPO1* mRNA, indicating inhibition of the XPO1 protein. In this group of heavily pretreated, relapsed, and refractory patients, seven of 15 evaluable patients (47%) achieved complete response or complete response with incomplete count recovery.

Conclusion

Selinexor, in combination with fludarabine and cytarabine, is tolerable at doses up to 55 mg/m^2 in pediatric patients with relapsed or refractory leukemia. All patients who received selinexor at ≥ 40 mg/m^2 demonstrated XPO1 target inhibition. Response rates are promising and will be further explored in a phase II trial.

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INTRODUCTION

Although survival rates are approximately 90% for children with acute lymphoblastic leukemia (ALL) and 60% for those with acute myeloid leukemia (AML), patients with relapsed or refractory disease have dismal outcomes.[1-4] However, because only a minority of pediatric patients have known targetable lesions, novel and broadly active agents are urgently needed. Selinexor (KPT-330), a selective inhibitor of exportin 1 (XPO1), may represent such an agent.

XPO1 is the primary nuclear exporter of key tumor suppressor and growth regulatory proteins, including p53, p73, p21, p27, NPM1, FOXO, and I-κβ.[5,6] Because the majority of these proteins require nuclear localization for their genome-surveying activities, increased nuclear export leads to decreased activity. Many malignancies, including AML, display elevated levels of *XPO1*.[5,6] Higher levels of *XPO1* are independently associated with a worse prognosis in adult patients with AML.[7] These data have led to the development of selective inhibitor of nuclear export (SINE) compounds

that covalently bind to the Cys528 residue of XPO1 and prevent binding of cargo proteins.[6] In preclinical models, SINE compounds are active in a wide variety of malignancies and may act synergistically with traditional chemotherapy.[8-16]

Selinexor is an orally bioavailable, slowly reversible SINE compound that specifically blocks XPO1 and undergoes hepatic metabolism and excretion. It has been studied extensively in preclinical models of T-lineage ALL and AML.[7,17-20] Selinexor causes apoptosis and differentiation in T-lineage ALL and AML cells lines, primary samples, and murine xenograft models, while sparing normal hematopoietic cells.[17,18] Activity was evident in xenograft models of AML cells, with on-target effects demonstrated by reduction in XPO1 protein and nuclear accumulation of p53 and NPM1.[17,20] In xenograft models, selinexor eliminated leukemia-initiating cells, as well as bulk tumor cells.[20] A recent phase I study demonstrated that selinexor can be safely administered as a single agent to adult patients with advanced solid tumors.[21] To our knowledge, this is the first report of selinexor in patients with hematologic malignancies, as well as the first report of selinexor given with conventional chemotherapy. We describe the safety, pharmacokinetics, pharmacodynamics, and activity of selinexor, in combination with fludarabine and cytarabine, in pediatric patients with relapsed acute leukemia.

PATIENTS AND METHODS

Patients

Patients with relapsed or refractory AML, ALL, myelodysplastic syndrome, or mixed phenotype acute leukemia (MPAL) who were ≤ 24 years old were eligible. Patients with AML, myelodysplastic syndrome, or MPAL were eligible if they were experiencing a first or subsequent relapse, whereas patients with ALL were eligible if they were experiencing a second or subsequent relapse. Refractory disease was defined as persistent disease after at least two courses of induction chemotherapy. Patients were required to have adequate hepatic and renal function, no evidence of graft-versus-host disease, and no uncontrolled infections. Patients could not receive investigational agents within 30 days of enrollment or myelosuppressive therapy within 14 days. Because selinexor is inactivated by glucuronidation and by conjugation with glutathione, acetaminophen was prohibited on days of selinexor administration. The protocol was approved by each site's institutional review board and registered at ClinicalTrials.gov (NCT02212561). Informed consent, and assent when appropriate, was obtained from all patients or their legal guardians.

Treatment Plan

Selinexor was given orally on days 1, 3, 8, 10, 22, and 24. Fludarabine (30 mg/m^2) and cytarabine (2 g/m^2) were given on days 15 through 19. Allowance was made to start fludarabine and cytarabine early for patients with progressive disease. Intrathecal (IT) chemotherapy was given before day 1. If CNS disease was present, IT therapy was given weekly until the cerebrospinal fluid became clear of leukemia. The starting dose of selinexor was 30 mg/m^2 (dose level 1), with planned dose escalations to 40 mg/m^2 (dose level 2), 55 mg/m^2 (dose level 3), and 70 mg/m^2 (dose level 4) in a rolling-six design.[22] Patients who had acceptable toxicity and clinical benefit were eligible to receive a second cycle of therapy. Supportive care included mandatory administration of dexamethasone, olanzapine, and ondansetron; voriconazole or posaconazole; and vancomycin plus ciprofloxacin or monotherapy levofloxacin during periods of neutropenia.

Definitions of Dose-Limiting Toxicities and Maximum Tolerated Dose

Toxicity was graded according to the Common Terminology Criteria for Adverse Events (version 4). Dose-limiting toxicities (DLTs) were based on nonhematologic toxicities that occurred during cycle 1 and were deemed possibly attributable to selinexor. Any grade 5 events and any grade 4 nonhematologic events possibly related to selinexor were considered DLTs, with the exception of electrolyte abnormalities reversible with standard intervention. Failure to receive at least 5 doses of selinexor by day 35 because of toxicity was considered a DLT. The maximum tolerated dose (MTD) was defined as the highest dose level at which one or fewer of six patients experienced a DLT.

Pharmacokinetic Studies

Blood samples were collected for pharmacokinetic (PK) analysis on days 1 and 22. On day 1, samples were collected before the dose; at 30 minutes; and at 1, 2, 4, 8, 24, and 48 hours after the dose. Samples were also collected before the 22-day dose and at 1, 2, 4, and 8 hours after the dose.

Pharmacodynamic Studies

To assess inhibition of XPO1, blood samples were collected before the first dose of selinexor and at 4, 8, 24, and 48 hours after the dose. Cells isolated from these samples were evaluated for mRNA expression levels by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of *XPO1*, which is upregulated in response to XPO1 inactivation. See Appendix (online only) for methods used for gene expression analysis.[9]

Response Criteria and Definitions

Response to single-agent selinexor was assessed by bone marrow aspiration on day 15 of cycle 1 unless fludarabine and cytarabine were started early because of progressive disease. Response to combination therapy was initially assessed between days 28 and 35 and reassessed at the time of count recovery. Patients were considered evaluable for response if they received at least five doses of selinexor. However, those who received fewer than five doses because of progressive leukemia were considered nonresponders. Measurement of response and minimal residual disease (MRD) were made on the basis of blast percentage by flow cytometry. Complete remission (CR) was defined as bone marrow with < 5% blasts, absolute neutrophil count (ANC) with ≥ 500 cells/μL, platelets with ≥ 75,000 cells/μL without transfusions, and no evidence of extramedullary disease. CR with incomplete blood count recovery (CRi) was defined as < 5% blasts, no extramedullary disease, but ANC < 500 cells/μL or platelet count < 75,000 cells/μL. Partial response was defined as bone marrow with 5% to 25% blasts and a decrease of at least 50% in blast percentage. MRD was defined as negative if the level was < 0.1%.

RESULTS

Patient Characteristics

Four patients had primary refractory disease at the time of enrollment, seven were experiencing a first relapse, and seven were experiencing a second relapse (Table 1). All patients who were enrolled while experiencing a first relapse had relapsed within 1 year of initial remission. Fifteen patients had AML, two had MPAL, and one had early T-cell precursor ALL after an initial diagnosis of AML. Ten patients had undergone prior stem-cell transplantation (SCT).

Table 1. Patient Characteristics and Treatment Outcomes

ID No.	Dose (mg/m^2)	Age (years)	Sex	Diagnosis	Disease Status	Prior SCT	Selinexor Doses Received	Day 0 MRD (%)	Day 15 MRD (%)	Final MRD (%)	Response to Combination Therapy	Subsequent SCT (days from enrollment)	Disease-Free Survival (months)
1	30	6	M	AML	2nd relapse	Yes	6	40	51	31	NR	No	NA
2	30	18	F	AML, t(6;12)	Refractory	No	2*	53	ND†	ND	NR	Yes (49)	NA
3	30	19	M	AML, −7	1st relapse	Yes	6	5	57	13	NR	Yes (63)	NA
4	30	6	M	MPAL	2nd relapse	Yes	6	59	12	0.35	CRi	Yes (96)	1
5	40	15	F	AML, t(6;9)	2nd relapse	Yes	6	43	< 0.1	< 0.1	CRi	Yes (90)	12‖
6	40	11	M	AML	2nd relapse	Yes	6	23§	39	< 0.1	CR	Yes (106)	12‖
7	40	17	M	AML, −7	1st relapse	Yes	6	16	15	< 0.1	CR	Yes (107)	11‖
8	55	2	F	AMKL	2nd relapse	Yes	6	5	19	26	NR	No	NA
9	55	4	F	MPAL, t(4;11)	2nd relapse	Yes	6	72	60	4.6	PR	No	NA
10	55	16	M	AML-M0	1st relapse	No	6	75	39	16	PR	Yes (98)	NA
11	55	5	F	AML, t(3;5)	1st relapse	No	6	28	10	21	NR	No	NA
12	55	16	F	AML, NK	Refractory	No	6	45	65	1.6	CR	Yes (80)	3‖
13	55	6	M	AML, t(9;11)	1st relapse	No	6	22	24	18	NR	Yes (62)	NA
14	70	13	F	AML, t(8;21)	1st relapse	No	6	30	28	<0.1	CR	Yes (99)	8‖
15	70	4	F	AML, NK	Refractory	No	6	16	<0.1	<0.1	CR	Yes (76)	6‖
16	70	5	M	AML	Refractory	No	4*	86‡	ND†	ND	NE	No	NA
17	70	13	M	AML/ETP	2nd relapse	Yes	6	90	82	ND	NE	No	NA
18	70	1.5	M	AML	1st relapse	Yes	4*	83	77	ND	NE	No	NA

Abbreviations: AMKL, acute megakaryoblastic leukemia; AML, acute myeloid leukemia; CR, complete response; CRi, complete response with incomplete blood count recovery; ETP, early T-cell precursor; ID, identification; MPAL, mixed phenotype acute leukemia; MRD, minimal residual disease; NA, not applicable; ND, not done; NE, not evaluable; NK, normal karyotype; NR, no response; PR, partial response; SCT, stem-cell transplantation.

*Patient No. 2 received only 2 doses because of progressive leukemia, whereas patients No. 14 and No. 16 received only 4 doses because of toxicity.

†Day-15 evaluation not preformed because patients began fludarabine and cytarabine early because of progressive disease. All other patients began fludarabine and cytarabine after day 15 evaluation.

‡Peripheral blood MRD.

§Estimated by morphology.

‖Disease-free survival ongoing.

Toxicity and MTD

Grade 3 and 4 nonhematologic toxicities occurring during cycle 1 are presented in Table 2. The most common Grade 3 nonhematologic toxicity related to selinexor was asymptomatic hyponatremia (median sodium nadir, 128.5 meq/L; range, 123 to 132 meq/L), which was easily corrected by oral or intravenous supplementation in all patients. Asymptomatic hypokalemia was also common.

All 16 patients who received fludarabine and cytarabine after initial dosing of selinexor developed pancytopenia. Three patients with persistent disease were not evaluable for time to count recovery because they began alternative chemotherapy on day 37, 43, and 45, respectively. Two patients began SCT conditioning on day 43 and 53, respectively. One patient (No. 17), with a history of two SCTs before enrolling in this trial, developed concurrent disseminated *Fusarium* fungemia and enterococcus faecium bacteremia on day 36 and had no follow-up after day 45 because he transitioned to palliative care. One patient had prolonged neutropenia before enrollment, which persisted after protocol therapy. Of the remaining 9 patients, the median time of ANC recovery to $\geq$ 500 cell/μL was protocol day 49. This translates to a median of 35 days (range, 21 to 47) after the start of fludarabine and cytarabine. The patients did not receive granulocyte colony-stimulating factor.

Table 2. Toxicity Profile of Patients

	Toxicity Grade							
	Dose Level 1		Dose Level 2		Dose Level 3		Dose Level 4	
Toxicity	3	4	3	4	3	4	3	4
Gastrointestinal disorders								
Nausea/vomiting			1					
Infections and infestations								
Febrile neutropenia	2		1		4		3	
Mucosal infection	1		1					
Anorectal infection							1	
Upper respiratory infection			1		1			
Lung infection	1				1		1	1
Meningitis	1							
Skin infection					1		1	
Infection, other							1	1
Metabolism/nutrition disorders								
ALT or AST increased	1		3		1		1	
Bilirubin increased	1							
Lipase increased							1	
Weight loss/anorexia					1		1	
Hyperglycemia					3		2	
Hyperkalemia	1							
Hypocalcemia	1				2			
Hypokalemia		3	1		3		3	
Hypomagnesemia			1					
Hyponatremia	3		2		3		4	
Hypophosphatemia	1		1		1		2	
Alkalosis							1	
Nervous system disorders								
Ataxia							2*	
Extremity pain			1					
Other								
Hypertension	2							
Hypotension							1	
Epistaxis			1		1		1	

NOTE. All grade 3 or 4 toxicities listed.
*Dose-limiting toxicity.

Two patients (No. 16 and No. 18; Table 1) experienced reversible cerebellar toxicity after receiving selinexor at 70 mg/m^2. The first patient (No. 16) was a 5-year-old male with refractory AML who had not undergone prior SCT. On day 23, after receiving four doses of selinexor and 5 days of fludarabine and cytarabine (without IT therapy), he had severe body pain, followed the next day by irritability, aphasia, and lower extremity weakness. Lumbar puncture showed normal opening pressure and no evidence of infection. Magnetic resonance imaging (MRI) showed diffuse cerebellar restricted diffusion with patchy restriction in the medulla and pons (Fig 1). He was empirically treated with intravenous immunoglobulin for 5 days, improved significantly over the next 3 weeks, and had resolution of symptoms after 6 weeks. A repeated MRI scan 10 days after the initial imaging showed nearly complete resolution of diffusion abnormalities. The second patient (No. 18) was a 19-month-old male with relapsed AML who had received prior SCT with busulfan, cyclophosphamide, and fludarabine conditioning. No CNS disease was detected at relapse. On day 13, after receiving four doses of selinexor and before receiving fludarabine or cytarabine, he became unable to hold his trunk or head straight and demonstrated erratic use of his hands. He had normal muscular tone and strength, but truncal instability when seated upright. An MRI scan revealed restricted diffusion and fluid-attenuated inversion recovery signal changes throughout the cerebellum (Fig 1). Lumbar puncture was deferred because of cerebellar edema seen on MRI. Because the clinical and imaging findings were consistent with cerebellar toxicity, protocol therapy was discontinued. His ataxia showed improvement over the course of 2 weeks. Neither patient had received radiation therapy, and neither had unusual difficulties with hyponatremia. No CNS toxicity was observed in any patients who received selinexor at doses $\leq$ 55 mg/m^2, thus establishing 55 mg/m^2 as the MTD.

Selinexor Pharmacodynamics and Pharmacokinetics

Plasma selinexor concentrations were assessed after the initial dose and after the day 22 dose. Mean plasma selinexor

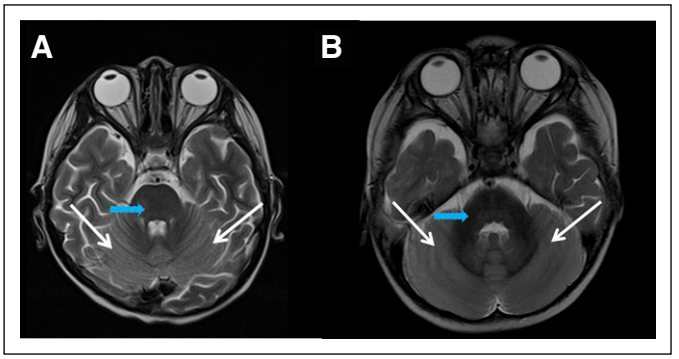

Fig 1. Axial T2-weighted magnetic resonance imaging scan of (A and B) the two patients with dose-limiting toxicity at 70 mg/m^2. Both patients show diffuse cerebellar edema and hyperintensity (white arrows), sparing the brainstem (blue arrows), deep cerebellar nuclei, and cerebrum.

concentration showed little separation between the 30- and 40-mg/m^2 dose levels, with an increase in exposure evident at the 55- and 70-mg/m^2 dose levels (Fig 2). The mean half-life of selinexor at 40, 55, and 70 mg/m^2 was 7.1, 7.6, and 7.2 hours, respectively. PK parameters measured on days 1 and 22 were comparable (Fig 2 and Appendix Table A1, online only). To assess the effects of selinexor on XPO1 protein, we used qRT-PCR to measure *XPO1* expression, which is upregulated at the RNA level in response to XPO1 protein inactivation.[9] Of the 16 patients assessed, 15 demonstrated at least twofold induction of *XPO1* mRNA (Fig 3). The only patient who did not achieve this level was receiving a dose of 30 mg/m^2. Increased expression persisted for at least 48 hours.

Leukemia Response

Bone marrow aspirations and biopsies were performed on day 15 of therapy to assess for early activity of single-agent selinexor, which was given on days 1, 3, 8, and 10. Two patients (No. 2 and No. 16) were not evaluable for single-agent selinexor response because fludarabine and cytarabine were started early because of progressive leukemia. At the early response assessment on day 15, two patients with AML, including one who had suffered a second relapse after SCT (No. 5) and one with primary refractory leukemia (No. 15), had CR with undetectable MRD (Table 1).

Although initial response to combination therapy was assessed between days 28 and 35, patients were predictably hypocellular at

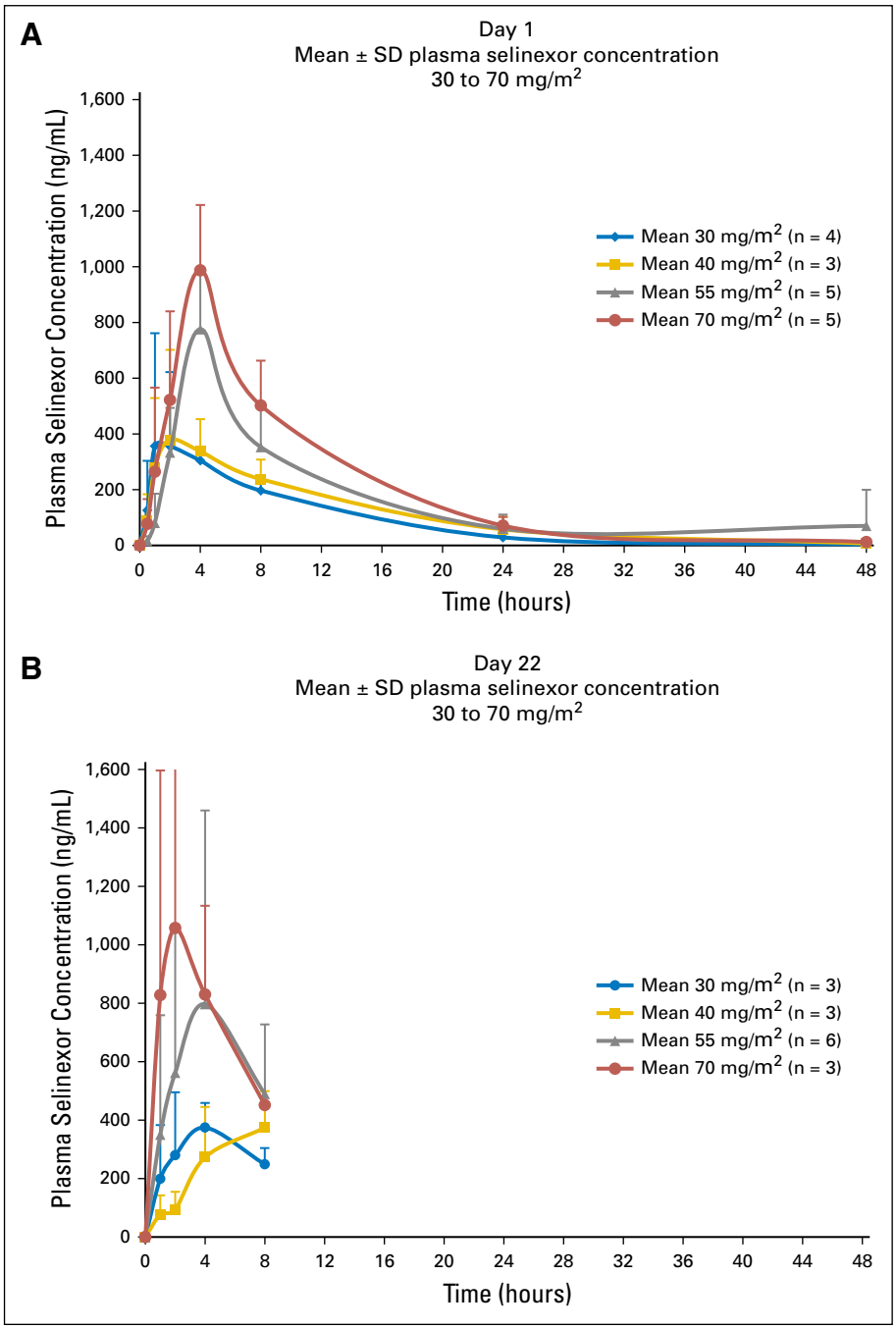

Fig 2. Mean plasma selinexor concentration in pediatric patients at day 1 showed little separation between the 30 and 40 mg/m^2 dose levels, with an increase in exposure evident at the 55 and 70 mg/m^2 dose levels. Mean plasma selinexor concentration in pediatric patients after the day-22 dose showed peak levels and dynamics similar to the day-1 levels.

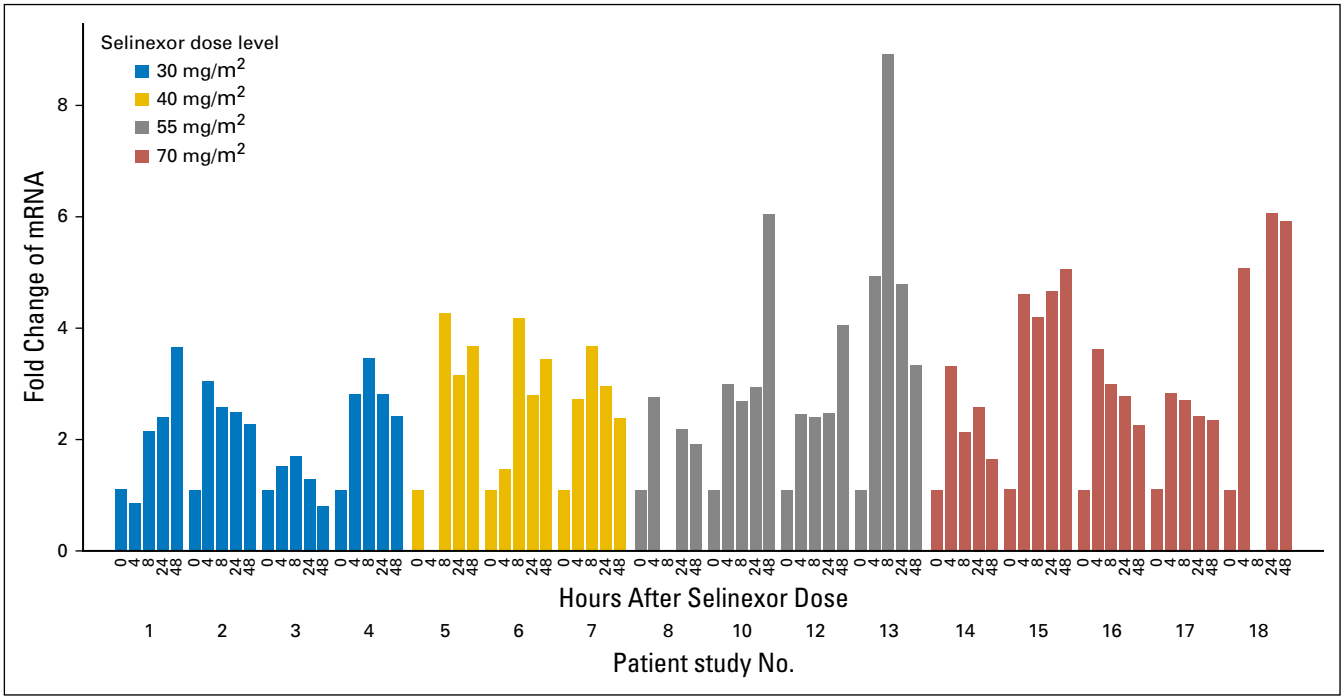

Fig 3. *XPO1* mRNA expression levels by dose. Inhibition of XPO1 was assessed by quantitative reverse transcription-polymer chain reaction of *XPO1*, which is upregulated at the RNA level in response to XPO1 protein inactivation.

this time. Therefore, evaluations of responses to combination (selinexor, fludarabine, and cytarabine) therapy were again performed when patients showed signs of hematopoietic recovery. Fifteen patients were evaluable for response to combination therapy. Two patients (No. 16 and No. 18) were not evaluable because they experienced DLTs before completion of cycle 1 and one patient (No. 17) because the family chose to pursue palliative care secondary to infectious complications before being evaluable for response. After the completion of one course of combination therapy, 7 of 15 evaluable patients (47%) achieved CR or CRi, including five who had no detectable MRD. All patients with CR/CRi subsequently underwent SCT (Table 1). Six of these seven patients were alive and in remission at a median time of 9.5 months (range, 3 to 12 months) from enrollment.

We incidentally observed that morphologic examination of the day-15 bone marrow aspirations showed clear evidence of myeloid differentiation in patients No. 5 and No. 14, despite the high day-15 level of MRD in patient No. 14 (Fig 4). The three patients (No. 5, No. 14, and No. 15) who demonstrated morphologic differentiation or flow cytometric complete response to single-agent selinexor at day 15 achieved a final response of CR or CRi, underwent SCT, and remain alive and in remission.

DISCUSSION

We report here the results of a phase I study to assess the safety, pharmacodynamics, pharmacokinetics, and activity of selinexor, the only inhibitor of nuclear export currently in clinical trials. Because preclinical[3,18-20] and phase I[21] data suggest that the DLTs of selinexor are anorexia, weight loss, and fatigue, we chose to give selinexor in combination with fludarabine and cytarabine, a commonly used regimen in which myelosuppression is the limiting toxicity. The treatment course included 2 weeks of single-agent selinexor, which allowed us to examine the safety and activity of selinexor alone in this pediatric population. Exposure to selinexor as a prephase may increase nuclear localization of many tumor suppressor proteins, which may augment the effectiveness of cytotoxic chemotherapy.[14]

Although data from a phase I trial of selinexor in adults with advanced solid tumors indicate that anorexia, weight loss, and fatigue are common, these adverse effects were rarely observed in this study.[21] The occurrence of hyponatremia and the need for sodium supplementation in nearly all patients highlight the need for frequent monitoring of serum electrolytes among patients treated with selinexor. The DLT, observed at 70 mg/m^2, was acute, severe, slowly reversible cerebellar toxicity. Cerebellar toxicity was also reported in one adult patient who received selinexor at a dose of 85 mg/m^2 in the recently reported phase I trial.[21] In our study, we cannot definitively attribute the cerebellar toxicity observed in the first patient to selinexor because this patient had also received fludarabine and cytarabine, which are known to cause neurotoxicity.[23,24] However, a second patient developed cerebellar toxicity characterized by similar symptoms and nearly identical MRI changes after receiving single-agent selinexor, suggesting that both occurrences represent dose-dependent toxicities.

Plasma concentration levels and measured half-lives in pediatric patients are similar to levels achieved in adults.[21] There were no significant differences in PK levels between day 1 and day 22 in our study, consistent with data in adults.[21] We did not

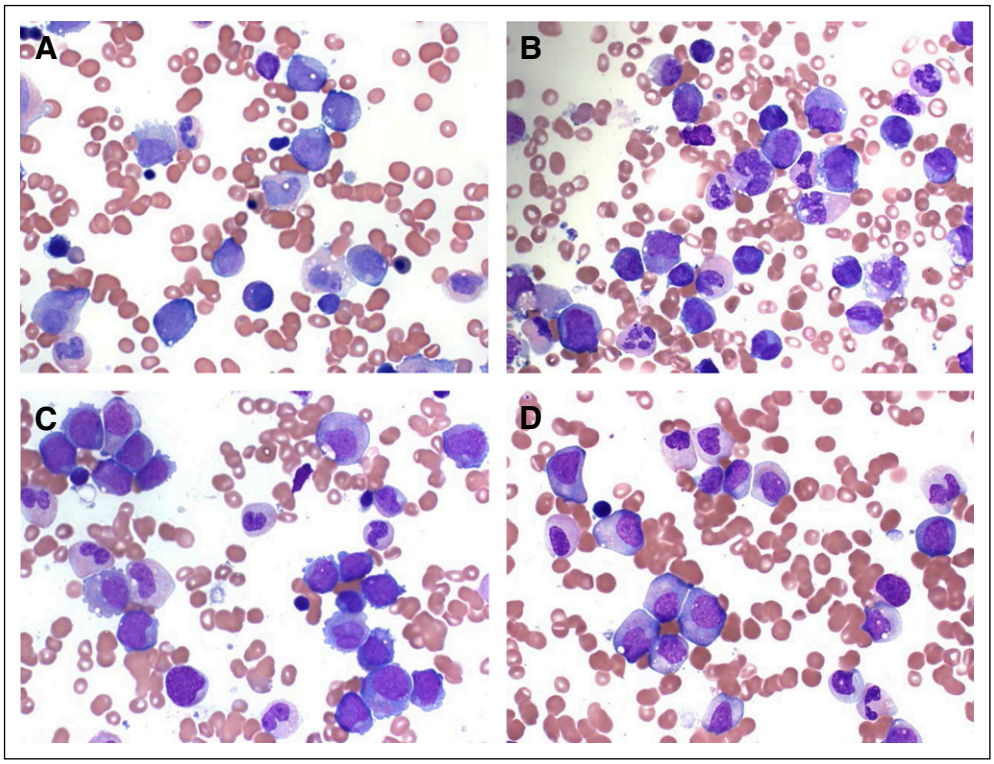

Fig 4. Bone marrow aspirates of patient No. 5: (A) before therapy, showing myeloblasts along with neutrophils, myelocytes, and promyelocytes; and (B) at day 15, showing differentiation evidenced by the absence of blasts, with an increase in neutrophils and myelocytes. Bone marrow aspirates of patient No. 14: (C) before therapy, showing monoblasts and occasional neutrophils; and (D) at day 15, showing a decrease in monoblasts with a corresponding increase in immature promonocytes.

observe differences in the PK levels between patients with or without DLT. Induction of *XPO1* mRNA expression, which was observed in all patients treated at $\geq$ 40 mg/m^2 and which was sustained for at least 48 hours, demonstrates on-target inhibition of XPO1 protein.[9] Although the ideal length of inhibition is not known, the PK and pharmacodynamic data suggest that the convenient dosing schedule used in this trial is sufficient to achieve target inhibition for at least 96 hours during the weeks of selinexor dosing.

The complete responses observed in this study suggest activity of the combined therapeutic regimen of selinexor, fludarabine, and cytarabine. Two recent large trials evaluating regimens for refractory or first relapse of pediatric AML showed complete response rates of 48% and 64%.[3,25] The higher-risk nature of our patient population and the small number of patients in the phase I portion of this study preclude direct comparisons with the response rates reported from these studies. The characteristics of our patient cohort more closely resemble those of patients enrolled in other early-phase trials for children with relapsed AML, such as a phase I trial of gemtuzumab ozogamicin and a phase II trial of clofarabine, in which response rates of 28% and 26%, respectively, were reported.[26,27] Notably, two patients in our study became MRD negative after receiving only selinexor, indicating that there is a patient population that might benefit from single-agent selinexor therapy. In addition, three of the seven patients who attained CR or CRi were in second relapse. Interestingly, the patient in our study with AML harboring the high-risk translocation t(6;9), which causes a *DEK-NUP214* chimeric protein, had an MRD-negative complete response to single-agent selinexor. The full role of this fusion protein in AML remains unclear, but NUP214 is a member of the nuclear pore complex and is the nucleoporin with the highest affinity for XPO1.[28-30] The relationship between selinexor-mediated XPO1 inhibition and *NUP214* fusions warrants further study. In addition to correlating response to selinexor regimens with cytogenetic or molecular leukemia subtypes, specific possibilities for analysis of predictive biomarkers include assessment of p53 mutational status, ex vivo treatment with selinexor followed by assays of apoptosis, or quantitation of *XPO1* transcript expression or XPO1 protein levels.[5,7,20]

In summary, to our knowledge, this is the first report of the use of selinexor in pediatric patients, as well as the first report of selinexor in patients with hematologic malignancies. We found that six doses of selinexor, given at 55 mg/m^2/dose, was well tolerated in combination with fludarabine and cytarabine in children with relapsed and refractory acute leukemia. The dose-limiting reversible cerebellar toxicity was only observed at a dose level of 70 mg/m^2. Complete responses observed in this high-risk population, after single-agent and combination therapy, suggest that selinexor may have a role in the treatment of pediatric acute leukemia. Taken together, this phase I study of selinexor, along with promising preclinical studies demonstrating efficacy in high-risk leukemia and on leukemia-initiating cells,[20] justifies further studies to evaluate the safety and efficacy of selinexor-based therapy in children with relapsed and refractory acute leukemia. In this regard, preclinical studies have shown that selinexor has synergistic activity when combined with agents such as DNA methyltransferase inhibitors[16] or MDM2 inhibitors,[7] suggesting that future trials include such combinations. We are currently planning a phase II study to further characterize the combination of selinexor, fludarabine, and cytarabine.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Data analysis and interpretation: Thomas B. Alexander, Norman J. Lacayo, Ching-Hon Pui, Jeffrey E. Rubnitz

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase I Study of Selinexor, a Selective Inhibitor of Nuclear Export, in Combination With Fludarabine and Cytarabine, in Pediatric Relapsed or Refractory Acute Leukemia

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Appendix

XPO1 Gene Expression Analysis Methods

Total RNA was isolated from whole-blood PAXgene samples (cat#762165; BD Biosciences, Sparks, MD) according to the manufacturer's protocol. cDNA was synthesized using high-capacity cDNA Reverse Transcription Kit (cat#4368813; Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. XPO1 mRNA levels were measured by quantitative polymerase chain reaction (QPCR) using predesigned TaqMan Gene Expression Assay (cat#Hs00185645; ThermoFisher). Each QPCR consisted of the following components: 0.5μL of XPO1 TaqMan assay, 0.5μl of GAPDH Control (cat#4326317E; Thermo Fisher Scientific), 5μL of Fast Advanced Master Mix (cat#4444963; Thermo Fisher Scientific), and 2μL water. QPCR was performed on the Viia7 System (Life Technologies, Carlsbad, CA), and results were analyzed using Excel (Microsoft, Redmond, WA).

Table A1. Selinexor Pharmacokinetics as a Function of Dose

Selinexor Dose (mg/m^2)	Day 1						Day 22			
	No. of Patients	C_{max} (ng/mL)	t_{max} (hours)	$AUC_{0\text{-}8h}$ (ng × h/mL)	$AUC_{0\text{-}48}$ (ng × h/mL)	$t_{1/2}$ (hours)	No. of Patients	C_{max} (ng/mL)	t_{max} (hours)	$AUC_{0\text{-}8h}$ (ng × h/mL)
30	4	537 ± 281	3 ± 1.5	2,171 ± 618	4,351 ± 513	6 ± 1	3	414 ± 124	4 ± 1	2,239 ± 494
40	3	475 ± 157	2 ± 3.5	2,311 ± 934	5,440 ± 940	7 ± 2	3	420 ± 87	4 ± 2	1,785 ± 376
55	4	776 ± 200	4 ± 0	5,663 ± 3,438	9,838 ± 2,413	8 ± 2.5	6	976 ± 665	3 ± 1	4,627 ± 2,484
70	5	996 ± 224	4 ± 1	4,986 ± 979	10,564 ± 1,638	7 ± 1	3	1,188 ± 474	2 ± 1	7,035 ± NA

Abbreviations: AUC, area under the curve; $C_{max,}$ maximum serum concentration; NA, not available; t_{max}, time to maximum serum concentration.