

Phase I and Pharmacologic Study of SNS-032, a Potent and Selective Cdk2, 7, and 9 Inhibitor, in Patients With Advanced Chronic Lymphocytic Leukemia and Multiple Myeloma

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A B S T R A C T

Purpose

SNS-032 is a highly selective and potent inhibitor of cyclin-dependent kinases (Cdks) 2, 7, and 9, with in vitro growth inhibitory effects and ability to induce apoptosis in malignant B cells. A phase I dose-escalation study of SNS-032 was conducted to evaluate safety, pharmacokinetics, biomarkers of mechanism-based pharmacodynamic (PD) activity, and clinical efficacy.

Patients and Methods

Parallel cohorts of previously treated patients with chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) received SNS-032 as a loading dose followed by 6-hour infusion weekly for 3 weeks of each 4-week course.

Results

There were 19 patients with CLL and 18 with MM treated. Tumor lysis syndrome was the dose-limiting toxicity (DLT) for CLL, the maximum-tolerated dose (MTD) was 75 mg/m², and the most frequent grade 3 to 4 toxicity was myelosuppression. One patient with CLL had more than 50% reduction in measurable disease without improvement in hematologic parameters. Another patient with low tumor burden had stable disease for four courses. For patients with MM, no DLT was observed and MTD was not identified at up to 75 mg/m², owing to early study closure. Two patients with MM had stable disease and one had normalization of spleen size with treatment. Biomarker analyses demonstrated mechanism-based PD activity with inhibition of Cdk7 and Cdk9, decreases in Mcl-1 and XIAP expression level, and associated CLL cell apoptosis.

Conclusion

SNS-032 demonstrated mechanism-based target modulation and limited clinical activity in heavily pretreated patients with CLL and MM. Further single-agent, PD-based, dose and schedule modification is warranted to maximize clinical efficacy.

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INTRODUCTION

B-cell malignancies such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) are characterized by accumulation of malignant cells resistant to apoptosis. Resistance to apoptosis is hypothesized to be due to overexpression of the Bcl-2 family of antiapoptotic proteins. Indeed, reduction in these proteins in vitro in primary CLL or MM cells through use of oligonucleotides^{1,2} or interference with their function using small molecule inhibitors³⁻⁶ resulted in rapid onset of apoptosis. The recognition that antiapoptotic Bcl-2 family members, such as Mcl-1, have short-lived transcripts and proteins, together with the observation

that some cyclin-dependent kinase (Cdk) inhibitors in clinical development potently inhibited Cdk9 and Cdk7, led to the strategy of transient inhibition of transcription as a means of initiating apoptosis in malignant B cells. Cdk9 and its cyclin partner, cyclinT1, are highly expressed and play important survival roles in CLL and MM.^{7,8} Indeed, inhibition of these Cdks by flavopiridol,⁹ SNS-032,¹⁰ or R-roscovitine¹¹ quickly reduced transcription, resulting in the rapid reduction of Mcl-1 and induction of apoptosis in primary CLL cells.

In addition to transcriptional control, the Cdk family of protein serine/threonine kinases includes key regulators of cell cycle progression.^{12,13} Cdk2 controls entry and progression through the DNA

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synthesis phase of the cell cycle and is altered in many cancer types.¹⁴ Aberrant expression of key regulators of Cdk2, such as cyclin E and p27, is associated with a poor prognosis and shorter survival in patients with cancer.¹⁵

Flavopiridol was the first Cdk inhibitor to enter clinical trial. Despite promising in vitro activity,^{16,17} initially, flavopiridol demonstrated no significant clinical activity in phase I/II studies in patients with relapsed CLL.^{18,19} Subsequent investigations revealed significant binding to human plasma proteins that altered free drug level, target cell exposure, and therefore therapeutic activity.^{20,21} Subsequently, a pharmacokinetic (PK)-derived schedule of a 30-minute intravenous bolus followed by 4-hour continuous infusion that sustained half maximal inhibitory concentration levels achieved a 50% response rate in patients with refractory CLL.^{22,23}

SNS-032 (BMS-387032) is a synthetic small-molecule inhibitor of Cdk2, 7, and 9 that exhibited potent in vitro cytotoxicity against cancer cell lines and primary cancer cells that was associated with down-modulation of Mcl-1.^{10,24,25} SNS-032 effectively killed primary CLL cells in vitro, independent of prognostic factor status or patient treatment history.¹⁰ This was associated with rapidly reversible inhibition of phosphorylation of RNA polymerase II (Pol II) and rapid downregulation of Mcl-1 and XIAP. SNS-032 was more potent at inhibiting RNA synthesis and inducing apoptosis than flavopiridol. Activity of SNS-032 was confirmed in vivo with human leukemia (HL-60 and MV 4-11) and MM (RPMI-8226) xenograft tumor mouse models.²⁵ Previously, three phase I trials of SNS-032 given as either 1- or 24-hour infusions to patients with metastatic refractory solid tumors or refractory lymphoma demonstrated tolerability, but did not show significant antitumor activity.^{24,26}

We conducted a phase I clinical study to assess the safety and tolerability of escalating doses of SNS-032, administered as a 5-minute loading dose (LD) followed by a 6-hour maintenance infusion weekly for 3 weeks of each 4-week course in patients with advanced CLL and MM. The dose schedule was based on PK data derived from previous clinical studies with SNS-032 aiming to maintain or exceed a target threshold plasma concentration of 115 ng/mL (0.3 μ mol/L) for 6 hours, which equals the concentration that inhibits 90% determined in in vitro assays.^{10,24,26}

PATIENTS AND METHODS

The trial was approved by the institutional review boards of all five participating institutions; all patients provided written informed consent. The study was conducted according to the Declaration of Helsinki.

Patients

Eligible patients had histologically confirmed CLL or MM; were \geq 18 years of age; had an Eastern Cooperative Oncology Group performance status of 0 to 2; and had adequate hepatic and renal function. Patients with CLL had measurable, relapsed disease after one or more prior treatment regimens. Patients with secretory MM had serum paraprotein more than 0.5 g/dL or 24-hour urine paraprotein more than 0.2 gm; patients with nonsecretory MM had detectable free serum light chain or plasmacytoma measurable by computed tomography or magnetic resonance imaging scan. Patients with MM also had two or more prior therapies, including (1) thalidomide, bortezomib, or lenalidomide and (2) autologous stem-cell transplantation. Patients with the following were excluded: prior SNS-032; thromboembolic event within 28 days; QT interval corrected for heart rate using Frederica's method more than 500 milliseconds; prior pelvic radiation or radiation to more than 25% of bone marrow reserve; or WBC count more than 200,000/ μ L, absolute neutrophil

count less than 500/ μ L, hemoglobin less than 8.5 g/dL, or platelet less than 30,000/ μ L.

Trial Design and Treatment Plan

The trial was planned as a two-stage, open-label, multicenter study of escalating doses of SNS-032 in parallel dose cohorts of patients with CLL and MM. SNS-032 was given as a 5-minute LD followed by a 6-hour infusion weekly for 3 consecutive weeks of each 4-week course. The primary end point was to determine dose-limiting toxicities (DLT) and identify a maximum-tolerated dose (MTD) for both cohorts. DLTs and MTD were identified during course 1; patients could continue courses if they did not experience DLT. Secondary end points were to estimate PK parameters and evaluate pharmacodynamic (PD) biomarkers.

Stage I was a standard 3 + 3 dose escalation according to Table 1. No dose modification was allowed during course 1. Dose delays were allowed for subsequent courses; patients must have received all three doses for course 1 within 28 days. Dose escalation occurred in the absence of protocol-defined DLT, until identification of the MTD. Adverse events (AEs) were identified and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. A DLT must have been an SNS-032 treatment-related event that occurred during course 1, including absolute neutrophil count less than 500/ μ L or platelet less than 30,000/ μ L lasting more than 14 days or not recovering to within 20% of baseline beyond day 28 of course 1; liver function test or electrolyte abnormality \geq grade 3 persisting more than 2 days; acute tumor lysis syndrome (TLS) requiring hemodialysis; any nonhematologic AE \geq grade 3; any abnormal nonhematologic laboratory value or AE requiring a dose delay of more than 14 days for recovery; inability to receive all three doses for course 1 within 28 days due to clinically significant abnormal laboratory values and/or nonhematologic AEs; or death. The MTD was defined as the dose at which fewer than two of six patients experienced DLT.

Prophylaxis for TLS was required for all patients with CLL for course 1, dose 1, and included admission to the inpatient service with cardiac monitoring, oral allopurinol 300 mg daily beginning 48 hours, and intravenous hydration beginning 24 hours before SNS-032 drug administration. For the first dose, vital signs were obtained before the 5-minute LD; at 30 and 60 minutes; at 2, 3, 4, 5, and 6 hours after starting the 6-hour maintenance infusion; at 1 hour after completing the 6-hour infusion; and thereafter every 2 hours until discharge. In the absence of TLS during the first dose, vital signs were obtained for all subsequent infusions before starting the 5-minute LD and 15 minutes after completing the 6-hour infusion. If TLS occurred anytime after the first dose, rasburicase could be given and TLS prophylaxis was required, regardless of TLS severity. If TLS did not occur with dose 1, prophylaxis could subsequently be omitted. Because TLS was not expected in MM, inpatient precautions were not mandated, but all patients with MM received intravenous hydration, oral allopurinol, and close outpatient monitoring with each dose.

Response to treatment was assessed before the start of each course and at the end of treatment. Response was assessed using 1996 National Cancer

Table 1. SNS-032 Dose Levels and Number of Patients Treated

Cohort	Dose (mg/m ²)			CLL (n = 19)	MM (n = 18)
	LD	6-Hour Infusion	Total		
1	5	10	15	0	3
2	10	12	22	2	1
3	10	23	33	5	4
4	10	40	50	3	5
5	10	65	75	6	5
6	10	90	100	3	0

Abbreviations: LD, loading dose; CLL, chronic lymphocytic leukemia; MM, multiple myeloma.

Phase I Study of SNS-032 in CLL and MM

Table 2. Patient Characteristics				
Characteristic	CLL (n = 19)		MM (n = 18)	
	No.	%	No.	%
Age, years				
Median	63		61	
Range	45-82		45-82	
Male sex		90		40
Performance status				
0	2		3	
1	16		13	
2	1		2	
Prior treatments				
Median	4		9	
Range	1-8		2-16	
WBC count, ×1,000/μL				
Median	6.9		3.5	
Range	2.4-112.5		1.8-7.2	
ANC, ×1,000/μL				
Median	1.7		2.4	
Range	0.7-12.6		0.8-5.4	
ALC, ×1,000/μL				
Median	2.3		0.8	
Range	0.7-105		0.3-2.7	
Platelets, ×1,000/μL				
Median	87.5		135	
Range	22-566		34-243	
Hemoglobin, g/dL				
Median	11.9		10	
Range	8.4-15		8.3-12	
β-2 microglobulin, mg/L				
Median	5.1		4.2	
Range	2.4-15.3		1.6-2812	
Prior stem-cell transplantation				
Allogeneic	2	11	1	6
Autologous			17	94
For patients with CLL				
Rai stage III to IV	13	68		
Fludarabine refractory	18	95		
Prior therapy				
FCR	10	53		
FR	5	26		
Alemtuzumab	6	32		
CD38 positive by flow	12	63		
FISH analysis, hierarchical, n = 13				
17p deletion	3	23		
11q deletion	2	15		
Trisomy 12	0			
None	4	31		
13q deletion	4	31		
For patients with MM				
Serum total protein, g/dL				
Median			8.9	
Range			5.8-14.2	
Serum M-spike, g/dL				
Median			3.8	
Range			0.14-8.8	
Prior therapy				
VAD			11	61
Dex/Thal			16	89
Bortezomib			16	89
Melphalan			9	50

Abbreviations: CLL, chronic lymphocytic leukemia; MM, multiple myeloma; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; FCR, fludarabine, cyclophosphamide, and rituximab; FR, fludarabine and rituximab; FISH, fluorescence in situ hybridization; VAD, vincristine, doxorubicin, and dexamethasone; Dex/Thal, dexamethasone, thalidomide.

Institute–Sponsored Working Group criteria²⁷ for CLL and European/International Bone Marrow Transplantation Registry guidelines²⁸ for MM. Patients with evidence of stable disease or better at the end of course 3 could receive three additional courses of treatment. Formal response assessment occurred every three courses, with continued treatment for stable disease or better.

PK

Blood samples were obtained on day 1 of course 1 at predose, end of LD, and 0.25, 0.5, 2, 4, 6, 8, 12, 24 and 30 hours after infusion. Plasma SNS-032 concentrations were determined by a validated liquid chromatography tandem mass spectrometry assay with a lower limit of quantification of 0.5 ng/mL. PK parameters were calculated by noncompartmental analysis using WTN-Nonlin (Scientific Consultant, Apex, NC) software, version 5.2 (Pharsight, Mountain View, CA).

PD

Mechanism-based biomarkers of SNS-032 PD activity were measured in blood mononuclear cells from 31 treated patients (19 with CLL) collected at the initial screen up to 1 hour before the 5-minute LD and at 2, 6, and 24 hours after starting the 6-hour maintenance infusion on course 1, day 1. The biomarkers evaluated were phosphorylation state of the C-terminal domain of the RNA Pol II; levels of the antiapoptotic proteins Mcl-1, Bcl-2, and XIAP; and cleavage of poly (adenosine diphosphate–ribose) polymerase (PARP) by immunoblotting as previously described in detail.¹⁰

RESULTS

Patient Characteristics

Characteristics of the 37 treated patients are summarized in Table 2. Patients in both cohorts were heavily pretreated, and virtually all patients with CLL (95%) had prior fludarabine therapy; 26% had fludarabine-refractory disease.

SNS-032 Dose Level and Associated Toxicities

The dose levels evaluated and number of patients treated at each level are summarized in Table 1. Patients with CLL received SNS-032 at 22 to 100 mg/m² total per dose. Biochemical evidence of TLS was observed in four patients with CLL treated at 75 mg/m², including one patient with grade 3 TLS. Grade 3 TLS was observed in two of three patients with CLL treated at 100 mg/m² and was the DLT; no patients required dialysis, and there were no treatment-related deaths. Therefore, the MTD identified for patients with CLL was 75 mg/m². SNS-032-related fever and fatigue were minimized with 100 mg of hydrocortisone pretreatment. Patients with MM received total SNS-032 from 15 to 75 mg/m² per dose. For patients with MM, the MTD was not established, and no DLTs were observed. Further enrollment and dose escalation was not continued as a result of early closure of the study. Biochemical TLS was not observed in any patient with MM as a result of low risk for TLS in this group.

SNS-032 infusions were well-tolerated; Table 3 lists all patients with grade 3 to 4 adverse events, related and unrelated, for all courses by diagnosis. Myelosuppression, including neutropenia, thrombocytopenia, and anemia, was the most frequently observed toxicity during treatment and seemed to be more common in patients with MM (Table 3). Other grade 3 to 4 adverse events were uncommon and sporadic across organ-system categories. Generally, similar proportions of patients experienced grade 3 to 4 adverse events between CLL (74%) and MM (78%). Appendix Table A1 (online only) reports all related and unrelated grade 1 to 2 adverse events that occurred in more than 10% of patients across all treatment courses. The most common

Table 3. All Grade 3 and 4 Toxicities Reported With SNS-032 Treatment

Adverse Events	CLL (n = 19)		MM (n = 18)	
	No. of Patients	%	No. of Patients	%
No. of patients reporting toxicity	14	74	14	78
Hematologic				
Neutropenia	3	16	7	39
Anemia	1	5	6	33
Thrombocytopenia	0	0	1	6
Febrile neutropenia	0	0	4	22
Gastrointestinal/hepatic				
Abdominal pain	2	11	0	0
Diarrhea	1	5	0	0
Vomiting	1	5	0	0
Cholecystitis	0	0	1	6
Hyperbilirubinemia	1	5	0	0
Transaminitis (ALT/AST)	2	11	0	0
Renal/electrolyte abnormal				
Acute renal failure	1	5	1	6
Hypokalemia	1	5	1	5
Hypocalcemia	1	5	0	0
Hypercalcemia	0	0	1	0
Hyperuricemia	1	5	0	0
Respiratory				
Hypoxia	2	11	0	0
Dyspnea	1	5	0	0
Lung infiltration	1	5	0	0
Cardiovascular				
Hypotension	2	11	0	0
Capillary leak syndrome	1	5	0	0
Nervous system				
Dizziness	1	5	0	0
Infection				
Pneumonia	2	11	2	11
Cellulitis	0	0	1	6
Sepsis	0	0	1	6
Streptococcal bacteremia	0	0	1	6
Otitis media	1	5	0	0
Other				
Asthenia	2	11	0	0
Fatigue/weakness	2	11	0	0
Noncardiac chest pain	1	5	0	0
Dehydration	1	5	0	0
Tumor lysis syndrome	4	21	0	0
Hyperglycemia	0	0	1	6

Abbreviations: CLL, chronic lymphocytic leukemia; MM, multiple myeloma.

grade 1 to 2 adverse events were GI-related, including nausea, vomiting, constipation, and diarrhea.

Treatment-associated prolongation of QTc interval occurred in nine and eight of the patients with CLL and MM, respectively; all were asymptomatic and not clinically significant. Prolongation of QTc was not dose-dependent. The maximal QTc after infusion was less than 450 milliseconds for all nine patients with CLL and included seven patients with prolongation less than 30 milliseconds, one patient with 30 to 60 milliseconds, and one patient with more than 60 milliseconds of prolongation. In the eight patients with MM with QTc prolongation, seven patients had prolongation of less than 30 milliseconds, and one patient had more than 60 milliseconds of prolongation. The

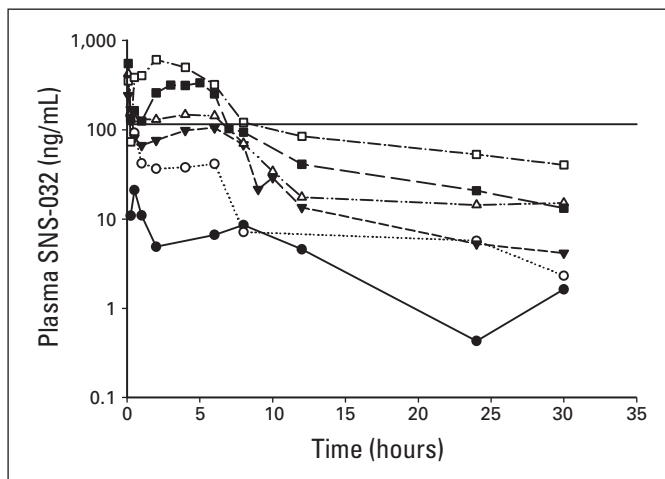


Fig 1. SNS-032 plasma concentration-time profiles. Mean pharmacokinetic profiles for all dose cohorts from patients with multiple myeloma and chronic lymphocytic leukemia are shown. Filled circles, 15 mg/m², n = 3; open circles, 22 mg/m², n = 2; filled inverted triangles, 33 mg/m², n = 8; open triangles, 50 mg/m², n = 7; filled squares, 75 mg/m², n = 10; open squares, 100 mg/m², n = 2; solid line, concentration that inhibits 90%.

maximal QTc after infusion was less than 450 milliseconds in five patients, between 450 and 480 milliseconds in two patients, and between 480 and 500 milliseconds in one patient with MM.

PK Analysis

Plasma concentrations of SNS-032 at different dose levels indicated that target SNS-032 concentrations (\geq in vitro concentration that inhibits 90%, 115 ng/mL, approximately 300 nmol/L, for at least 6 hours) were achieved and exceeded with the 50 mg/m² dose level (cohort 4) with maximum plasma concentration at 6 hours of 142.7 ng/mL (Fig 1). The maximum plasma concentration values at 6 hours were 251.5 and 319 ng/mL for the 75 mg/m² (cohort 5) and 100 mg/m² (cohort 6) dose levels, respectively (Table 4). These concentrations decreased rapidly to less than 115 ng/mL on completion of the 6-hour infusion. Greater than dose-proportional increase in exposure (area under the concentration versus time curve from time zero to infinity) was observed in both patients with CLL and MM, in contrast to prior studies of SNS-032 administered to patients with advanced solid tumors, in which PK values were dose proportional.²⁶

PD Analysis

PD studies were possible in patients with CLL owing to the availability of circulating leukemia cells. The decrease in phosphorylation of Ser5 and Ser2 in the C-terminal domain of RNA Pol II during infusion of 75 mg/m² total dose (Fig 2A) was indicative of inhibition of Cdk9 and Cdk7 by SNS-032. Inhibition was seen as early as 2 hours after starting the SNS-032 infusion and was pronounced after 6 hours, with a return to baseline after 24 hours. The inhibition of pSer2 (64%) was more significant than for pSer5 (35%) at 6 hours after SNS-032 infusion, consistent with a more potent half maximal inhibitory concentration for Cdk9 inhibition as compared with that for Cdk7 (4 v 62 nmol/L).

Transcriptional inhibition by SNS-032 was shown by marked downregulation of Mcl-1 protein level (56% at 6 hours) and modest downregulation of XIAP protein level (10% at 6 hours and 23% at 24 hours; Fig 2B). There was no significant decrease in Bcl-2 protein level after SNS-032 infusion. Apoptosis was detected by PARP cleavage seen 6 hours after the start of infusion. Similar target modulation was observed for patients 1504 (75 mg/m²), 1601, and 1602 (both at 100 mg/m²; Fig 2C), with more pronounced increase in PARP cleavage at 100 mg/m² (not shown). A complete set of results were available for four of the nine patients with CLL at the 75 mg/m² and 100 mg/m² dose levels, as shown in Figure 2C. Results for three additional samples showed decreases in Mcl-1 expression, and seven additional samples showed cleavage of PARP after SNS-032 infusion. The consistency of these observations in evaluable samples indicates that these patterns are representative of the response of CLL to SNS-032.

Antitumor Activity of SNS-032

Overall, there was limited evidence of antitumor activity for SNS-032. For patients with CLL, one patient in cohort 4 (75 mg/m²) had a short-lived decrease in measurable disease, but anemia and thrombocytopenia did not improve to qualify as a partial remission by 1996 National Cancer Institute–Sponsored Working Group response criteria. The patient was taken off study after course 4 with progressive disease. Another patient in the same cohort had stable disease for four courses, with a small decrease in tumor volume. The patient was taken off study after course 5 on patient request. One patient with MM in cohort 3 (33 mg/m²) had stable disease after three courses. Another patient with MM in cohort 4 (75 mg/m²) had normalization of spleen

Table 4. Pharmacokinetic Parameters

Dose (mg/m ²)	No. of Patients	AUC _{INF} (h × ng/mL)		CL (h × ng/mL)		C _{max 6 h} (ng/mL)		T _{1/2} (hours)		MRT _{INF} (hours)		V _{ss} (L/m ²)	
		Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
15	3	376	22	41.22	23	38.13	17	8.68	23	8.42	23	348	34
22	2	630		36.74		41.5		7.39		5.73		229	
33	8	1,099	16	30.74	17	105.1	22	8.39	41	5.3	39	165	45
50	7	1,668	37	35.12	51	142.7*	19	9.77	75	7.89	78	228	43
75	10	2,767	25	28.71	26	251.5	47	9.02	30	6.55	31	185	34
100	2	6,049		18.12		319		15.95		14.23		194	

Abbreviations: AUC_{INF}, area under the concentration versus time curve from time zero to infinity; CL, total body clearance; C_{max}, maximum plasma concentration; T_{1/2}, terminal half-life; MRT_{INF}, mean residency time when the drug concentration-time profile is extrapolated to infinity; V_{ss}, apparent volume of distribution at steady-state; CV%, percent coefficient of variation.

*n = 6.

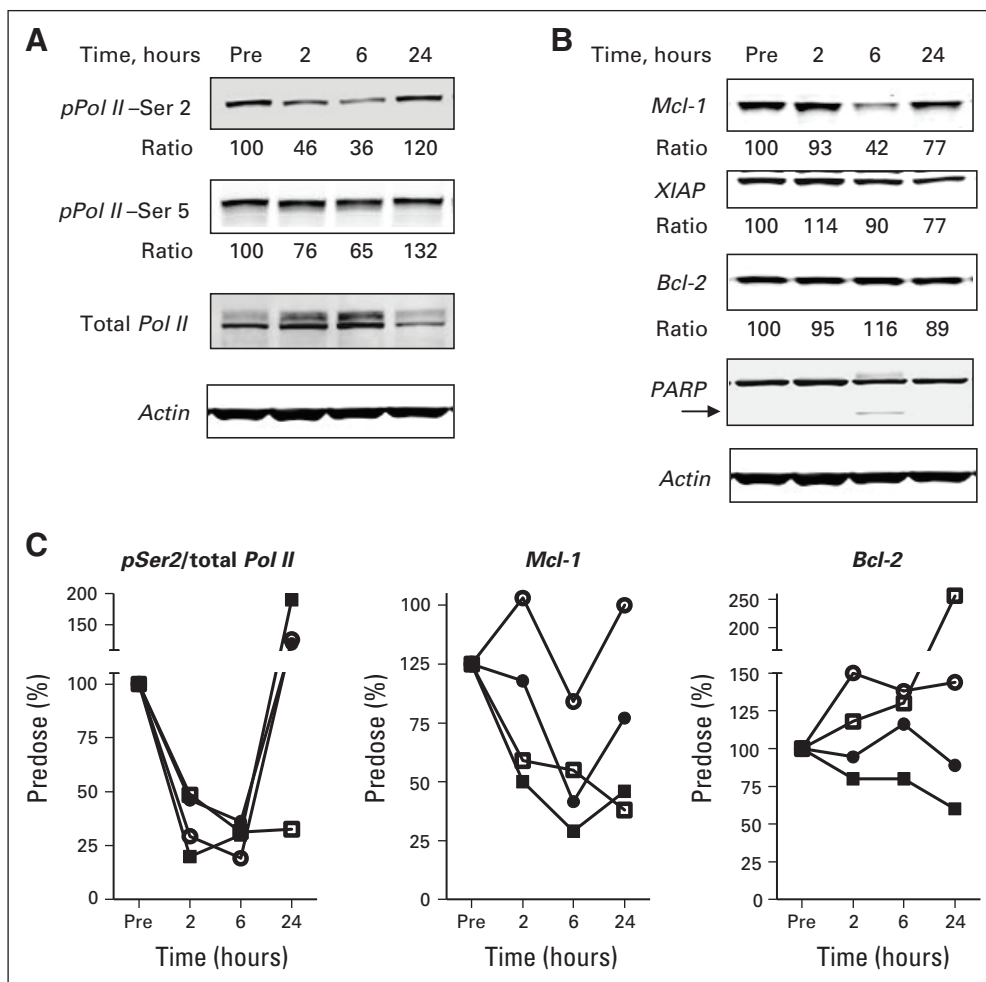


Fig 2. Target modulation after SNS-032 infusion in patients with chronic lymphocytic leukemia. (A) Representative immunoblots from a patient treated with 75 mg/m² of SNS-032. The ratio indicates the densitometry analysis between pPol II-Ser2 or pPol II-Ser5 and total Pol II, normalized to predose controls. (B) Representative immunoblots showing the protein levels of Mcl-1, XIAP, and Bcl-2 after SNS-032 infusion. The ratio indicates the densitometry analysis between Mcl-1, XIAP or Bcl-2, and actin, normalized to predose controls. Cleavage of poly (adenosine diphosphate-ribose) polymerase (PARP; arrow) was used as an indicator of apoptosis. (C) The summarized quantitations of phosphorylation of Ser2 at the C-terminal domain of RNA Pol II and protein levels of Mcl-1 and Bcl-2 for two patients treated with 75 mg/m² (closed symbols) and two patients treated with 100 mg/m² (open symbols) of SNS-032.

size and stable disease, and the patient was taken off study after course 5 on the basis of investigator decision.

DISCUSSION

This phase I and pharmacology study was conducted to assess the safety and tolerability of SNS-032 in patients with advanced CLL and MM and to relate these findings to the PK and PD of this agent. For patients with CLL, the DLT was TLS, and the MTD was 75 mg/m². The DLT and MTD were not identified for patients with MM because the study was closed early. PK results from this study indicated that target plasma levels were exceeded and sustained for 6 hours in patients who received SNS-032 doses of 50 mg/m² and greater. In CLL, pharmacodynamic studies demonstrated mechanism-based activity against the target Cdks that was associated with decreased levels of antiapoptotic proteins and CLL cell death.

In vitro studies with SNS-032 and primary CLL cells demonstrated reversible inhibition of Cdks with exposures for less than 10 hours and removal of SNS-032 from the culture medium after treatment for \leq 6 hours permitted rephosphorylation of RNA Pol II, which led to re-expression of Mcl-1 protein and cell survival.¹⁰ Indeed, rephosphorylation of RNA Pol II was observed in patient samples at a time when plasma SNS-032 had decreased below

target levels (Fig 2). Therefore, longer exposure to the target plasma concentration of 115 ng/mL may be required to maximize antitumor activity, which could be achieved by lengthening the infusion duration.

The limited clinical efficacy of SNS-032, despite observing TLS and potent in vitro activity with target modulation, could be explained by several reasons. First, the biochemical TLS involves not only the death of CLL cells, but other cell types. However, there were no significant electrolyte abnormalities, symptoms, or indications of TLS in patients with MM at doses studied, making this an unlikely explanation. Second, in vitro studies showing reversible inhibition of Cdks by SNS-032 suggested that target levels may need to be maintained for \geq 8 hours and that the 6-hour infusion may have been insufficient for antitumor effects. Third, patients in this study were heavily pre-treated; clinical responses with an improved therapeutic index may be observed in patients with earlier-stage disease.

Overall, SNS-032 was well tolerated by most of the treated patients, with grade 3 to 4 neutropenia and thrombocytopenia as the most common toxicities. Serious infections were infrequent with antibiotic prophylaxis. Patients who developed TLS did not require hemodialysis, and no mortality was associated with TLS, although aggressive prehydration and prophylactic monitoring for TLS were used in this trial.

SNS-032 works by a p53-independent mechanism of action, making it a potentially relevant drug in treating patients with CLL and chromosome 17p deletion, a high-risk feature for which there are limited treatment options. It also blocks vascular endothelial growth factor, thereby inhibiting angiogenesis and potentially contributing to cytotoxicity.²⁹ Rational combinations with SNS-032 for patients with CLL may include an inhibitor of protein synthesis, such as homoharringtonine, to affect a sequential blockade of the antiapoptotic protein expression.³⁰ A previous study with roscovitine, a small-molecule Cdk inhibitor, confirmed its synergistic effect with alemtuzumab and restored alemtuzumab sensitivity in alemtuzumab-resistant CLL cells.³¹

In conclusion, SNS-032 is a highly selective and potent inhibitor of Cdk2, 7, and 9. In this phase I trial, single-agent SNS-032 demonstrated mechanism-based target modulation as well as modest clinical activity in heavily pretreated patients with CLL and MM. The dose schedule of a loading dose followed by a 6-hour infusion achieved the predicted plasma concentrations and was well-tolerated. The most commonly observed toxicity was myelosuppression, although TLS was observed in patients with CLL treated at ≥ 75 mg/m², which was managed conservatively without dialysis or fatalities. The schedule of single-agent SNS-032 could be further optimized by longer duration of exposure at doses that achieve the threshold plasma level and warrants further clinical study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about

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