

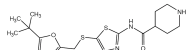
# Mechanism of Action of SNS-032, a Novel Cyclin Dependent Kinase Inhibitor, in Chronic Lymphocytic Leukemia: Comparison with Flavopiridol

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## Introduction

- Cyclin dependent kinases (Cdks) not only drive cell cycle progression, but also control transcription. For example, Cdk7 and Cdk9 phosphorylate the Ser2 and Ser5 sites, respectively, on the C-terminal domain (CTD) of RNA polymerase II (pol II) to promote transcription initiation and elongation.
- Our previous experience with flavopiridol, a pan-Cdk inhibitor, in primary chronic lymphocytic leukemia (CLL) cells demonstrated inhibition of RNA pol II-driven mRNA synthesis, reduction of the expression of short lived anti-apoptotic proteins, and induction of apoptosis in CLL cells in vitro (Blood 106:2513-19, 2005).
- Here we studied a novel Cdk inhibitor SNS-032, a 2-aminothiazole derivative with potent and selective inhibitory activity against Cdk2, Cdk7 and Cdk9.



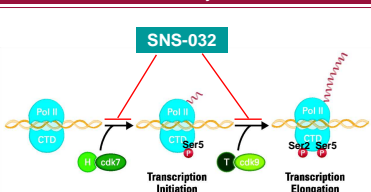
SNS-032

- SNS-032, formerly known as BMS-387032, is currently in a Phase 1 trial in patients with chronic lymphocytic leukemia and multiple myeloma. SNS-032 is administered using a pharmacologically-derived dose regimen, designed to sustain levels above 0.3 µM for over 6 hours, the EC<sub>50</sub> concentration and time required for in vitro cytotoxicity.

## Hypothesis

Because CLL cells are not actively cycling, and their viability is dependent upon the continuous expression of anti-apoptotic proteins, we hypothesized that SNS-032 would induce apoptosis in CLL cells through transcriptional inhibition of anti-apoptotic proteins.

### Activation of RNA Pol II by Cdk7 & Cdk9 and Inhibition by SNS-032



Modified from Shapiro, G. L. J Clin Oncol; 24:1770-1783, 2006

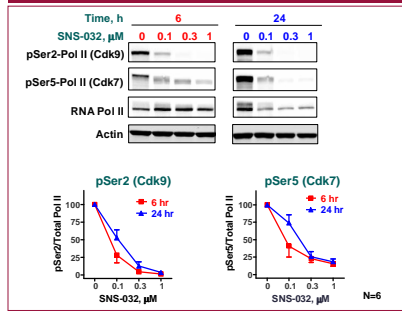
### Inhibition of Cdks by SNS-032 and flavopiridol

Kinase	SNS-032 IC <sub>50</sub> (nM)	Flavopiridol IC <sub>50</sub> (nM)
Cdk1/cyclin B	480	41 <sup>a</sup>
Cdk2/cyclin A	38	100 <sup>b</sup>
Cdk4/cyclin D	925	65 <sup>c</sup>
Cdk5/p35	340	~100 <sup>c</sup>
Cdk6/cyclin D	>1000	~100 <sup>c</sup>
Cdk7/cyclin H	62	~300 <sup>c</sup>
Cdk9/cyclin T	4	6 <sup>d</sup>

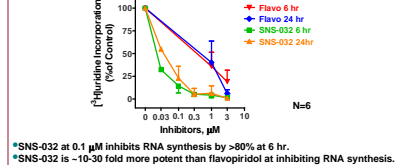
<sup>a</sup>Losiewicz et al.(1994)Biochem. Biophys. Res. Commun. 201:589; <sup>b</sup>Carlson et al.(1996)Cancer Res 56:2973; <sup>c</sup>Senderowicz and Sausville(2000)NCL 92:376; <sup>d</sup>Chao et al. (2000) JBC 275, 28345.

## Results

### SNS-032 inhibits the phosphorylation of RNA pol II in CLL cells

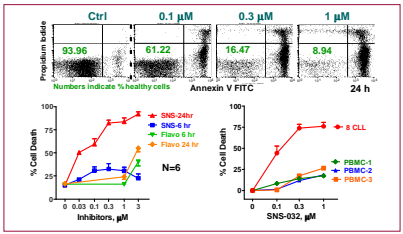


### SNS-032 inhibits RNA synthesis

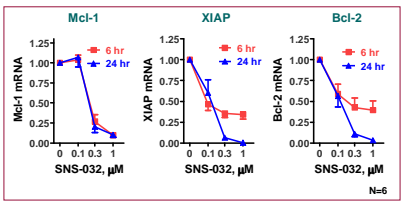


<sup>a</sup>SNS-032 at 0.1 µM inhibits RNA synthesis by >80% at 6 hr.  
<sup>b</sup>SNS-032 is ~10-30 fold more potent than flavopiridol at inhibiting RNA synthesis.

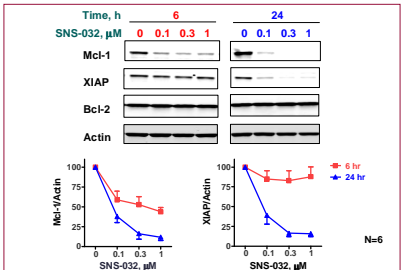
### SNS-032 potently induces apoptosis in CLL cells



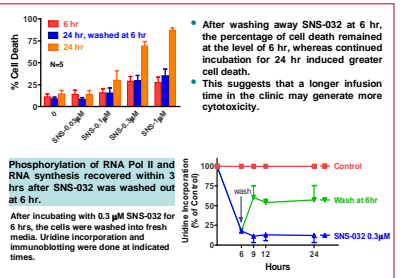
### SNS-032 reduces transcripts of anti-apoptotic proteins in CLL cells



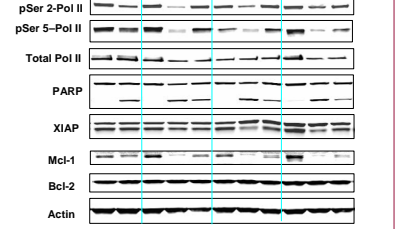
### Reduction of anti-apoptotic protein levels by SNS-032



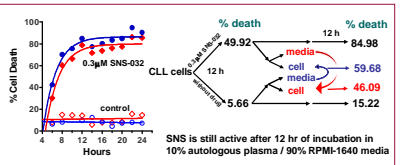
### Recovery of CLL cells after washing into fresh medium without SNS-032



### SNS-032 induced cell death is not dependent on a functional p53 or ATM



### Time course of SNS-032 induced apoptosis



### SNS-032 induced cell death is not dependent on a functional p53 or ATM

CLL Pt	Control 24 h	SNS 0.3 µM 24 h	Net	p53 deletion	ATM deletion
1	6.9	91	84		
2	6.1	84	78	X	X
3	16	91	65		
4	15	63	68		
7	15	77	62		
8	2.8	51	46		X
9	18	69	51	X	
10	8.9	68	59		
12	27	82	55		
13	18	67	49	X	
14	21	74	53		
15	18	88	70		
22	23	71	48		
23	20	70	50		
24	15	86	71		
25	7.3	90	83		
26	10	62	51		
27	20	61	41		
28	15	85	70		
Mean	15	76	61		

Red numbers identify the six patient samples in the first 5 figures in Results.  
Blue numbers identify the additional two patient samples in the experiment comparing toxicity of SNS-032 on CLL versus normal lymphocytes.

## Summary

- SNS-032 inhibits the phosphorylation of Cdk7/Cdk9-substrates Ser5/Ser2 of CTD of RNA Pol II, and reduces RNA synthesis.
- SNS-032 reduces the mRNA and protein levels of short-lived anti-apoptotic proteins Mcl-1 and XIAP.
- SNS-032 induces apoptosis in CLL cells.
- Killing of CLL cells by SNS-032 is maximal between 10 to 12 hr exposure.
- Induction of apoptosis appears independent of p53 or ATM.
- SNS-032 is about 10-30-fold more potent than flavopiridol.

## Conclusion

These in-vitro results demonstrate mechanism-based cytotoxicity of SNS-032 in human CLL cells and support the ongoing Phase 1 clinical study of SNS-032 administered to patients with chronic lymphocytic leukemia or multiple myeloma.

