

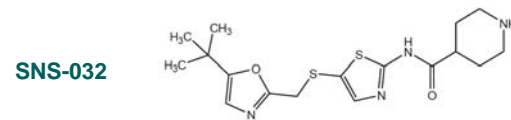
SNS-032, a novel inhibitor of cyclin-dependent kinases 2, 7 and 9, blocks transcription of cyclin D1 and Mcl-1, causing cell death in mantle cell lymphoma cell lines

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Introduction

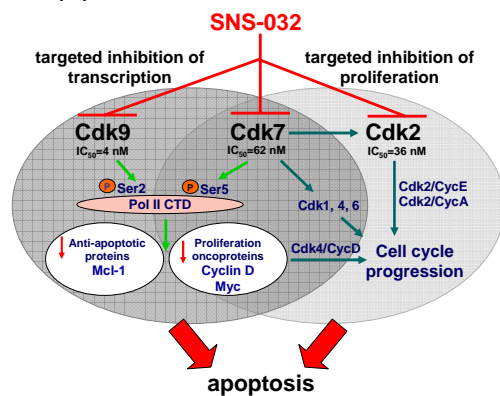
- Cyclin dependent kinases (Cdks) not only drive cell cycle progression, but also control transcription. For example, Cdk7 and Cdk9 phosphorylate the Ser5 and Ser2 sites, respectively, on the C-terminal domain (CTD) of RNA polymerase II (pol II) to promote transcription initiation and elongation.
- The novel Cdk inhibitor SNS-032, a 2-aminothiazole derivative, has potent and selective inhibitory activity against Cdk2, Cdk7 and Cdk9.



- Mantle cell lymphoma (MCL) is an aggressive form of non-Hodgkin lymphoma. It is characterized by the chromosomal translocation t(11:14)(q13;q32), which results in over-expression of cyclin D1, with the consequent deregulation of cell cycle control at the G1/S checkpoint.
- Many transcription factors (Myc, Jun, etc.), cell cycle control proteins (cdk4, RB, etc.), apoptotic control proteins (Bcl-2, etc.) are also over-expressed in MCL cells.
- The transcripts and expressed proteins of cyclin D1, Mcl-1 and Myc are all short-lived, therefore are ideal disease-associated targets for transcription inhibition, and thus provide a biological context for SNS-032 therapy in MCL.

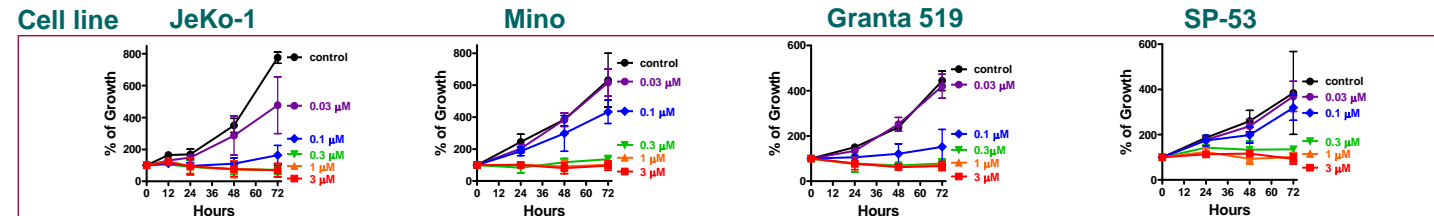
Hypothesis

We hypothesized that the malignant phenotype of MCL is sustained by the continued expression of short lived oncoproteins, and that transient inhibition of transcription will cause a critical decrease in such proteins and initiate apoptosis.

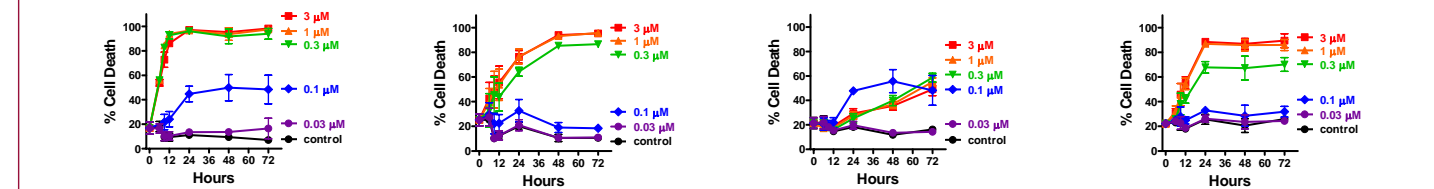


Results

Inhibition of growth

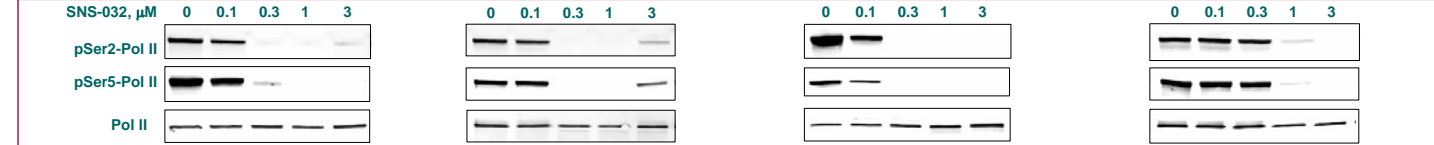


Induction of apoptosis (annexin V/PI staining)

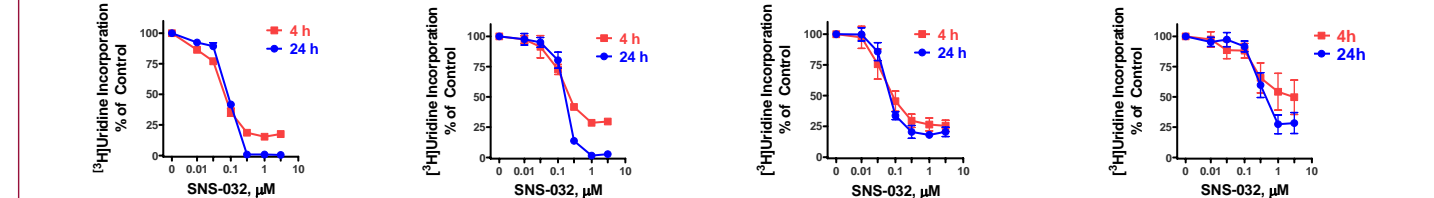


Inhibition of RNA synthesis

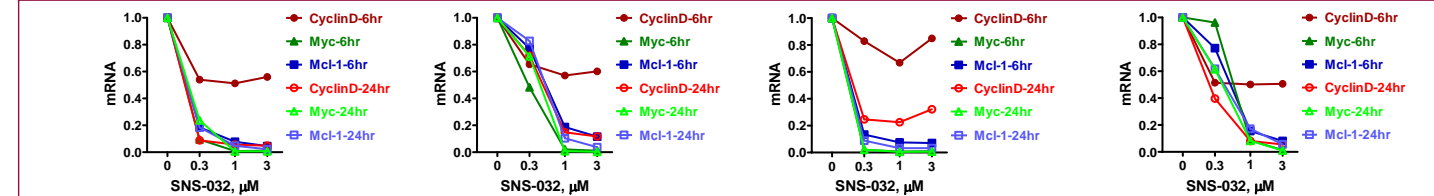
RNA Pol II phosphorylation 24 h



[3H]uridine incorporation

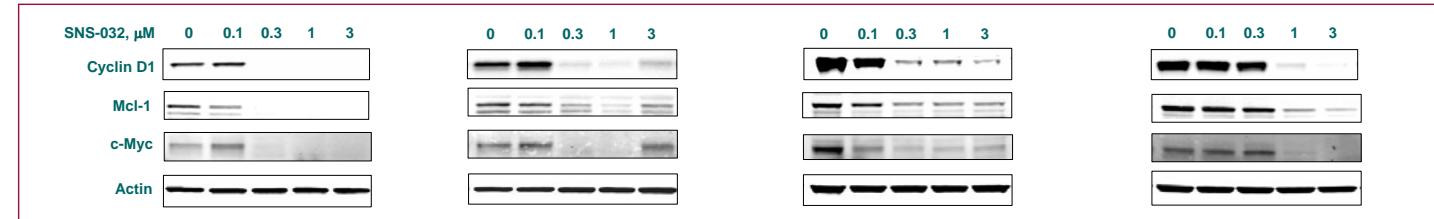


Reduced mRNA of oncoproteins



Reduction of oncogenic proteins

24 h



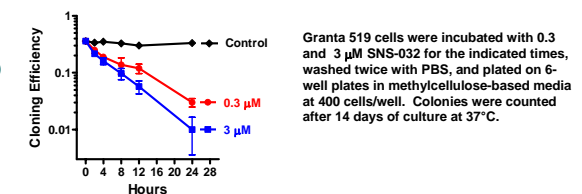
Comparing the four MCL cell lines

	JeKo-1	Mino	Granta 519	SP-53
Cyclin D1	+	+	+	+
Rb	+	+	+	+
p16	+	+	-	-
p21	+	+	+	+
p27	+	+	+	+
p53	+	+	+	+
TP53 gene	del/mut ^a	mut, exon5	del/wt ^a	wt
ATM	wt, amp ^a		mut ^a	

Ref: Amin, AM et al, Arch Pathol Lab Med 127:424-431, 2003
^a Camp J et al., Leuk Res 30: 923-934, 2006

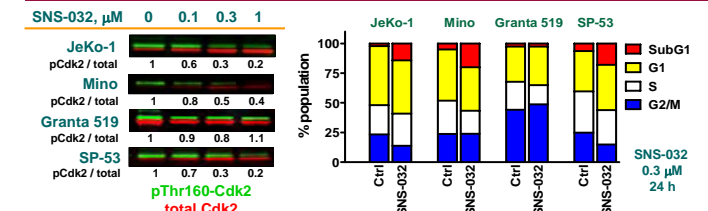
SNS-032 inhibits cell repopulation capacity

Granta 519



Granta 519 cells were incubated with 0.3 and 3 μM SNS-032 for the indicated times, washed twice with PBS, and plated on 6-well plates in methylcellulose-based media at 400 cells/well. Colonies were counted after 14 days of culture at 37°C.

Effect of SNS-032 on cell cycle



Summary

- SNS-032 inhibited the phosphorylation of the Cdk7/Cdk9-substrates, Ser5/Ser2 of CTD of RNA Pol II. This was associated with a reduction of RNA synthesis in MCL lines.
- SNS-032 reduced the mRNA and protein levels of short-lived oncoproteins Mcl-1, Myc and cyclin D1.
- Cell growth and clonogenicity in MCL cells were decreased by SNS-032.
- Induction of apoptosis varied in dose and timing with cell line. Concentrations of ≥ 0.1 or 0.3 μM SNS-032 induced apoptosis that appears to be independent of p53.
- Growth inhibition and cytotoxicity by SNS-032 were not associated with perturbations in cell cycle distribution in the MCL cell lines.
- A Phase 1 clinical study of SNS-032 administered to patients with chronic lymphocytic leukemia or multiple myeloma is currently ongoing.

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