Responses in Mantle Cell Lymphoma Cells to SNS-032 Depend on the Biological Context of Each Cell Line

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Abstract

SNS-032 is a potent inhibitor of cyclin-dependent kinases (Cdk) 2, 7, and 9 that regulate the cell cycle and transcription. Our studies in indolent primary chronic lymphocytic leukemia cells showed that SNS-032 inhibited transcription, diminished the antiapoptotic protein Mcl-1, and induced apoptosis. The present study focuses on evaluating this compound in four proliferating mantle cell lymphoma lines (Jeko-1, Granta 519, Mino, and SP-53). Consistent with its action against Cdk9 and Cdk7, SNS-032 inhibited the phosphorylation of RNA pol II in all four lines and blocked RNA synthesis. The transcripts and protein levels of short-lived proteins decreased, including cyclin D1 and Mcl-1. Cell growth was inhibited in a concentration-dependent manner in all lines. Apoptosis was induced in JeKo-1, Mino, and SP-53 cells without disrupting cell cycle distribution. However, apoptosis was limited in Granta cells; rather, there was a significant reduction of clonogenic survival. Small interfering RNA was used to specifically knock down Mcl-1 and cyclin D1 in JeKo-1 and Granta cells. Knocking down Mcl-1 induced significant apoptosis in Jeko-1 cells but not Granta cells. Reducing cyclin D1, rather than Mcl-1, was associated with loss of clonogenic survival in Granta cells. Thus, these results indicated that mantle cell lymphoma cell lines have distinct mechanisms sustaining their survival, and the mechanism of action of SNS-032 is dependent on the biological context of an individual line. Cancer Res; 70(16); 6587-97. ©2010 AACR.

Introduction

Mantle cell lymphoma (MCL) is an aggressive subtype of non–Hodgkin lymphomas that constitutes 5% to 10% of patients with the disease (1, 2). It is the result of a malignant transformation of B lymphocytes in the outer edge of a lymph node follicle, called the mantle zone. MCL is genetically characterized by the t(11;14)(q13;q32) translocation that juxtaposes the proto-oncogene CCND1, which encodes cyclin D1, at chromosome 11q13, to the immunoglobulin heavy chain gene at chromosome 14q32. This translocation leads to the constitutive overexpression of cyclin D1, which is not detected in normal lymphocytes. As cyclin D1 couples with cyclin-dependent kinases (Cdk) and regulates the transition of cells from the G1 to S phase of the cell cycle, this overexpression was thought to contribute to uncontrolled growth of the disease (1). Despite the response rates of 50% to 70% with current regimens of chemotherapy and immunotherapy, the disease typically progresses after treatment. The median survival time is ~3 years; the 10-year survival rate is only 5% to 10%. Thus, MCL remains incurable with current therapeutics and awaits more effective treatment approaches (1–3).

SNS-032 (formerly BMS-387032) is a potent Cdk inhibitor for a select group of Cdns with Ki values in the nanomolar range (4). It has been evaluated both in vitro (4, 5) and in clinical trials against advanced B-cell malignancies (6) and solid tumors (7). Originally selected as an inhibitor of Cdk2 (IC50, 38 nmol/L; ref. 4), the compound was later found to be a potent inhibitor of Cdk9 (IC50, 4 nmol/L) and Cdk7 (IC50, 62 nmol/L; ref. 4). The Cdns that regulate the initiation and elongation of transcription by phosphorylating Ser2 and Ser5 sites in the tandem repeat of RNA polymerase II (pol II) COOH-terminal domain (CTD), respectively. Recent investigations showed the actions of SNS-032 as an inhibitor of transcription in chronic lymphocytic leukemia (CLL) cells, an indolent disease model that does not exhibit cell cycle progression (5).

In the present study, we postulated that SNS-032 would be a unique and active compound in MCL, a highly proliferative disease, based on the following rationale: (a) The inhibition of transcription will eliminate the short-lived antiapoptosis protein Mcl-1 and induce apoptosis (5). (b) Transcriptional inhibition will also reduce cyclin D1 levels, which would affect proliferation. Cyclin D1 mRNA is transcriptionally upregulated in MCL cells. However, both
the mRNA and protein of cyclin D1 turn over rapidly. Although some variation of cyclin D1 transcripts has been described, >90% of cases reported have the AUUUA sequence in the 3′-untranslated portion of the transcript that predisposes the transcript for rapid degradation (8). (c) Direct inhibition of Cdk2 by SNS-032 would inhibit cell cycle progression. (d) Inhibition of Cdk7, which together with Mat, also functions as the Cdk-activating kinase by phosphorylating Cdk1, 2, 4, and 6, in concert with the downregulation of cyclin D1, would block cell cycle progression. Our results in four MCL cell lines showed that the inhibition of transcription by SNS-032 causes a profound reduction of the cellular proteins. Elimination of the antiapoptotic protein Mcl-1, rather than cyclin D1, was responsible for apoptosis in Jeko-1, Mino, and SP-53 cells. In contrast, reduction of cyclin D1 and inhibition of Cdk2 may contribute to the diminished clonogenic survival in Granta 519 cells.

Materials and Methods

Materials
SNS-032 was provided by Sunesis Pharmaceuticals, Inc. It was prepared as a 10 mmol/L stock solution in DMSO and stored at −20°C in small aliquots. [3H]uridine (50 Ci/mmol) was purchased from Moravek Biochemical, Inc. Annexin V-FITC Apoptosis Detection kit was purchased from BD Biosciences. Propidium iodide (PI) solution (1 mg/mL) was purchased from Sigma Aldrich.

Cell lines
The four MCL cell lines (Granta 519, Jeko-1, Mino, and SP-53; ref. 9) used in this study were kindly provided by Dr. Hesham Amin of the University of Texas M.D. Anderson Cancer Center, Houston, TX. The cells were maintained in DMEM with 20% fetal bovine serum (FBS; Granta-519), RPMI 1640 with 10% FBS (Jeko-1), and RPMI 1640 with 20% FBS (Mino and SP-53), respectively (10). Cell lines were authenticated by STR DNA fingerprinting using the AmpFISTR Identitiﬁer kit (Applied Biosystems). The STR proﬁles were compared with known American Type Culture Collection fingerprints to the Cell Line Integrated Molecular Authentication database version 0.1.200808 (http://bioinformatics.istge.it/clima/; ref. 11) and to the German Collection of Microorganisms and Cell Cultures database (http://www.dsmz.de/). The STR proﬁles of Jeko-1, Mino, and Granta 519 matched known DNA fingerprints. The STR proﬁle of SP-53 cells was unique.

Growth inhibition
MCL cells were seeded on 24-well plates at 5 × 10⁴/mL; SNS-032 was added the next day. Cell concentrations were measured at 24, 48, and 72 hours after addition of SNS-032 by Cell and Particle Counter (Beckman Coulter, Inc.). Data were presented as percentage of control cell growth.

Quantitation of cell death
Cell death after SNS-032 treatment was evaluated by flow cytometry analysis using Annexin V and PI double staining according to the manufacturer’s instruction (Becton Dickinson). Samples were analyzed with a Becton Dickinson FACSCalibur flow cytometer (Becton Dickinson). Data acquisition and analysis were performed by the CellQuest program. Cells stained positive for either Annexin V or PI were considered dead cells.

Clonogenic assay
After incubating the cells with SNS-032 for 2 to 24 hours and washing them with PBS, or 6 hours after transfection with the small interfering RNAs (siRNA), Granta cells were mixed with methylcellulose media (Stemcell) with 5 mmol/L glutamine, 1% bovine serum albumin, 30% FBS, and 0.5% glucose at the density of 400 cells/mL, and dispensed to 12-well plates at 1 mL per well in triplicates. Colonies (aggregates composed of >50 cells) were counted after 14 days of culture at 37°C. Clonogenic efficiency was ~30%.

RNA synthesis
RNA synthesis was measured by quantitating incorporation of [3H]uridine into the perchloric acid–insoluble materials as previously described (12).

Immunoblot analysis
Cells were collected and lysed by sonication, and the lysates were subjected to immunoblotting as described before (12). The blots were scanned by an Odyssey Infrared Imaging system (LI-COR Biosciences) to obtain images and quantitations. The antibody to Mcl-1 (S-19) was purchased from Santa Cruz Biotechnology. The antibody to poly ADP ribose polymerase (PARP) was from Biomol International, Inc. Antibodies for total RNA Pol II (8WG16), phosphorylated CTD at Ser2 (H5) or Ser5 (H14), were purchased from Covance Research Products, Inc. pT821 retinoblastoma protein (Rb) and nucleophosmin (NPM) antibodies were purchased from Invitrogen. Antibody for cyclin D1 was from BD Biosciences, which recognize the A isoform of cyclin D1. Antibodies for pS807/811-RB, total Rb, and pT199-NPM were from Cell Signaling Technology. Actin antibody was purchased from Sigma Aldrich. Alexa Fluor 680 goat anti-mouse IgG was purchased from Invitrogen. IRDye 800CW Goat Anti-rabbit IgG was from LI-COR Biosciences.

RNA isolation and real-time quantitative reverse transcription-PCR
Total cellular RNA was isolated by the RNeasy kit from Qia-gen. The mRNA levels were measured by real-time quantitative reverse transcription-PCR (RT-PCR) as previously described (5). The relative gene expression was analyzed by the Comparative Ct method using 18s rRNA as endogenous control. The results were presented as the percentage of gene expression of the time-matched controls. All primers, probes, and reaction mix were purchased from Applied Biosystems.

Cell cycle analysis
Exponentially growing MCL cells were incubated with SNS-032 for 24 hours. About 5 × 10⁵ cells were collected, washed with ice-cold PBS, and fixed in 2.5 mL 70% ethanol overnight. Fixed cells were washed twice with cold PBS
before incubation in 300 μL PBS with 15 μg/mL PI and 2.5 μg/mL DNase-free RNase A (Roche) for 30 minutes at 4°C. Fluorescence was measured by flow cytometry.

**siRNA transfection**

The On Target Plus Smartpool siRNAs specific for Mcl-1, cyclin D1, and c-Myc were purchased from Dharmacon, Inc. Granta 519 and Jeko-1 cells were transfected with 1.5 μg siRNA with Nucleofector 1 (Amaxa) using the Cell Line Nucleofector kit V and program D-23 for Granta 519 cells and T-02 for Jeko-1 cells. Six hours after transfection, Granta 519 cells were seeded for clonogenic assay as described above. At 24 and 48 hours after transfection, cells were collected for viability assay and immunoblotting. Transfection efficiency was measured by flow cytometry using a plasmid expressing the green fluorescent protein (Amaxa). Transfection efficiency was ∼60% for both cell lines.

**Statistical analysis**

Statistical analysis was carried out by the Student’s t test using the GraphPad Prism software (GraphPad Software, Inc.). A P value of <0.05 was considered to be statistically significant.

### Results

**SNS-032 inhibited cell proliferation and induced cell death in MCL cells**

Toxicity of SNS-032 in the MCL cells was first evaluated by measuring cell growth after addition of SNS-032 (Fig. 1A). SNS-032 at a range of 0.03 to 3 μmol/L induced a concentration-dependent inhibition of cell growth. Although sensitivity varied, 0.3 μmol/L SNS-032 was maximally effective in all cell lines. The IC_{50} values of inhibition of cell growth after a 72-hour incubation with SNS-032 were similar in Jeko-1 (0.06 ± 0.04 μmol/L, mean ± SD) and Granta 519 cells (0.06 ± 0.02 μmol/L), followed by Mino (0.12 ± 0.02 μmol/L) and SP-53 cells (0.14 ± 0.02 μmol/L).

As inhibition of cell growth represents the combined effect of blocking cell cycle progression and induction of cell death, we studied both aspects of SNS-032 in the MCL cells. Apoptosis was induced rapidly in Jeko-1 cells in as soon as 6 hours and reached a maximum at 12 hours by 0.3 μmol/L SNS-032 (Fig. 1B). The pattern was similar in Mino and SP-53 cells, except that they were relatively less sensitive. In contrast, Granta 519 cells were resistant to SNS-032-induced apoptosis. There was very little cell death at 24 hours after SNS-032, even at concentrations >0.3 μmol/L, whereas the same concentrations clearly inhibited cell growth (Fig. 1A). These data indicated that the major effect of SNS-032 on Granta cells is blocking proliferation. Clonogenic assays were used to further investigate the effect of SNS-032 on the cell repopulating capacity (Fig. 1C). Granta 519 cells were incubated with 0.3 μmol/L SNS-032 for 2, 4, 8, 12, and 24 hours before they were washed and seeded in semisolid media. There was a time-dependent inhibition on clonogenicity in which 4 hours of exposure to SNS-032 resulted in 50% reduction in the number of colonies.

**SNS-032 inhibited RNA synthesis and reduced the expression of short-lived oncoproteins**

Previous studies showed that transcriptional inhibition of antiapoptotic proteins is a key mechanism for Cdk9 inhibitor–induced cell death in indolent B-cell malignancies (5, 13). To evaluate if a similar mechanism was active in proliferating MCL cells, we studied the phosphorylation status of the Pol II CTD after a 6- and 24-hour exposure to increasing concentrations of SNS-032 (Fig. 2A). Phosphorylation of both Ser2 (Cdk9 site) and Ser5 (Cdk7 site) was inhibited by SNS-032 at 6 hours. There was less inhibition of Ser5 phosphorylation in all cell lines, consistent with a higher Ki in the inhibition of Cdk7 (62 nmol/L) compared with Cdk9 (4 nmol/L; ref. 4). The inhibition of Pol II phosphorylation was associated with the decrease of total RNA synthesis, measured by [3H]uridine incorporation at both 6 and 24 hours (Fig. 2B). The IC_{50} values for inhibition of RNA synthesis after a 24-hour incubation were 0.08 ± 0.01 μmol/L (mean ± SD) for Jeko-1 cells, 0.08 ± 0.02 μmol/L for Granta 519 cells, 0.16 ± 0.03 μmol/L for Mino, and 0.66 ± 0.11 μmol/L for SP-53 cells. Similar to the effect on growth inhibition, Jeko-1 and Granta 519 cells were more sensitive to SNS-032 than were Mino and SP-53 cells.

Because both Mcl-1 and cyclin D1 oncoproteins have rapid turnover rates, we expected that their transcripts and protein levels would decrease upon the inhibition of transcription. A real-time RT-PCR analysis showed a concentration-dependent reduction of Mcl-1 mRNA by >90% at 0.3 μmol/L for Jeko-1 and Granta 519 cells (Fig. 3A). In Mino and SP-53 cells, 1 μmol/L SNS-032 was required to achieve a 90% reduction. There was little difference in Mcl-1 mRNA level between 6 and 24 hours. Cyclin D1 mRNA was reduced moderately after 6 hours and was further reduced at 24 hours, consistent with a relatively longer mRNA half-life (Fig. 3B). The protein levels of Mcl-1 were also reduced after only 6 hours of incubation (Fig. 3C) when all cell lines except Granta 519 initiated apoptosis, as indicated by cleaved PARP. Cyclin D1 protein levels were decreased in all cell lines after a 24-hour exposure to SNS-032. There was a renewed expression of Mcl-1 and cyclin D1 at 24 hours compared with 6 hours at 0.1 μmol/L SNS-032, which did not completely inhibit transcription. This reversal may reflect a compensatory stress response under conditions of partial inhibition of transcription. Alternatively, cells with relatively low Mcl-1 may have been killed by this time, whereas those with greater levels may have survived for analysis. Proapoptotic proteins such as Bim and Noxa have been implicated in MCL pathogenesis (14, 15). Bim was not detected in Jeko-1, Mino, and SP-53 cells likely due to homozygous deletions (14). In Granta cells, there was no change in Bim expression after SNS-032 treatment (Supplementary Fig. S1). We did not observe substantial changes in the level of Noxa in any of the cell lines.

**Effect of SNS-032 on cell cycle**

In contrast to a previous report of SNS-032–induced G2 arrest in HCT-116 cells (2), there was no significant cell cycle arrest in Jeko-1, Mino, and SP-53 cells 24 hours after incubation with SNS-032 (Fig. 4A). There was, however, an increase...
in cells with a sub-G1 DNA content, indicating dead cells. In Granta 519 cells, there was no change in the small sub-G1 population. Rather, there was a subtle increase in G1 and G2-M population, and a decrease in cells in S phase. We observed substantially more Annexin V-positive cells than sub-G1 population in samples of the same treatment, possibly because Annexin V detects an early stage of apoptosis, whereas sub-G1 cells are in late stages of cell death during which the DNA has been fragmented and substantially lost from the cells. The effect of SNS-032 on cell cycle-regulating proteins and kinases was investigated by immunoblotting. First, we studied the phosphorylation of Cdk2 by the CAK activity of Cdk 7. There was no significant change of Cdk2 phosphorylation at Thr160, indicating that the Cdk7 CAK activity was not affected by SNS-032 (Fig. 4B). Next, we investigated the phosphorylation status of Rb protein. Upon phosphorylation by Cdk4/6/cyclin D, and further by Cdk2/cyclin E, Rb dissociates from E2F, which activates the expression of proteins needed for S-phase entry and progression. In Jeko-1, Mino, and SP-53 cells, Rb phosphorylation on S807-811 (Cdk4/cyclin D1 substrate), as well as pT821 Rb (Cdk2/cyclin E substrate), was reduced 6 hours after incubation with SNS-032 and was further decreased after 24 hours. However, there was a coincident and profound decrease in the total Rb level, which correlated with cell death and PARP cleavage. Additional immunoblotting with the G3-245 antibody showed a caspase cleavage product p100 Rb at 6 hours after SNS-032 exposure (Supplementary Fig. S1), consistent with the suggestion that such processes may contribute to the decrease of Rb protein (16). On the contrary in Granta 519 cells,
in which Rb protein level remained relatively stable, there was a consistent decrease in phosphorylated Rb/total Rb protein, indicating inhibition of kinase(s) responsible for Rb phosphorylation.

NPM is a multifunctional protein involved in ribosome biosynthesis, stress response, and maintenance of genomic stability (17). Upon phosphorylation by Cdk2/cyclin E at threonine 199 in early G1 phase, NPM dissociates from centrosomes, which triggers centriole separation and centrosome replication (17). After SNS-032 treatment, NPM protein appeared stable in all cell lines. However, inhibition of NPM phosphorylation was apparent in JeKo-1, Mino cells, and moderately in Granta cells, indicating loss of Cdk2 activity.

In addition to Rb, we also observed a general decrease of other cell cycle–regulating proteins, such as the replication initiation protein Cdc6 and the Cdk regulator p27, in all four cell lines (Supplementary Fig. S1). There was no apparent change in the expression of Cdk inhibitor p21, consistent with a previous report in JeKo-1 cells after flavopiridol treatment (18). Both Rb and p27 are tumor suppressor proteins that inhibit cell cycle progression. Eliminating these proteins by SNS-032 would facilitate cell cycle transition, which counteracts the inhibition of Cdk5 and the reduction of Cdc6. The lack of a prominent cell cycle arrest after SNS-032 treatment may be due to the simultaneous activation of these antagonistic processes.

**Effect of siRNA depletion of Mcl-1 and cyclin D1 on short- and long-term cell survival**

Reducing Mcl-1 expression was associated with SNS-032–induced cell death in CLL cells. As SNS-032 reduced the expression of both Mcl-1 and cyclin D1, siRNA was used to differentiate the contribution of each protein in cell survival and proliferation. SiRNA against Mcl-1 reduced the Mcl-1 protein level by ~80% in JeKo-1 (Fig. 5A and B) and Granta 519 cells (Fig. 6A and B). There was a significant reduction of cell viability in JeKo-1 cells 24 hours (data not shown) and 48 hours (Fig. 5C) after the transfection. However, reduction of Mcl-1 had no effect on cell viability in Granta 519 cells (Fig. 6C), indicating that Mcl-1 is not required for maintaining Granta cell viability. Cyclin D1 expression was reduced by its siRNA by 50% in each cell line with no change in cell viability (Figs. 5C and 6C).
Reducing both Mcl-1 and cyclin D1 levels in JeKo-1 cells did not induce more cell death than Mcl-1 siRNA alone. These results indicated that cyclin D1 is not a survival factor for any of the cell lines. However, clonogenic assays showed that reducing cyclin D1, rather than Mcl-1, was associated with a significant reduction of clonogenic survival in Granta (Fig. 6D), suggesting that depletion of cyclin D1 may contribute to the growth arrest and loss of clonogenicity in these cells.

**Discussion**

This study investigated the actions of a potent Cdk 2, 7, and 9 inhibitor, SNS-032, in MCL cell lines. Our results
showed that SNS-032 was active against all four lines studied. However, its mechanism of action seems to be dependent on the biological context of the disease. In JeKo-1, Mino, and SP-53 cells, which are dependent on the expression of the anti-apoptosis protein Mcl-1 for survival, transcription inhibition of Mcl-1 by SNS-032 induced a rapid apoptosis without perturbing cell cycle distribution. In Granta 519 cells in which Mcl-1 is dispensable for viability, apoptosis was not induced. Rather, the long-term proliferating potential was inhibited after a transient exposure to the SNS-032, associated with the inhibition of Cdk2 and suppression of cyclin D1 expression. These results indicated that Cdk inhibition by SNS-032 results in a differential downstream response in different cell lines, predicated on the intrinsic properties of that line. Likely, this differential sensitivity reflects heterogeneity in MCL lines derived from different sources.

Figure 4. Effect of SNS-032 on cell cycle. A, SNS-032 did not disturb the cell cycle distribution in the four MCL cell lines after incubation with 0.3 μmol/L SNS-032 for 24 h. B, effect of SNS-032 on Cdk2, Rb, and NPM phosphorylation. Phosphorylation status was analyzed by immunoblotting with antibodies specific for the Cdk2 activation site by Cdk7 (pT160), pS807/811-Rb (Cdk4/cyclin D1 site), and pS821-Rb (Cdk2/cyclin E site), as well as pT199-NPM (Cdk2/cyclin E site). The fluorescence intensity was quantitated and normalized to level of total protein, setting values for nontreated cells as 1.
Although the MCL is genetically characterized by the t(11;14)(q13;q32) and the overexpression of cyclin D1, deregulation of cyclin D1 in transgenic mouse models is not sufficient for lymphoma development (19, 20). Thus, additional oncogenic events that either drive cell cycle progression or alter the apoptosis pathway may cooperate with cyclin D1 overexpression in lymphomagenesis. In this study, SNS-032 reduced cyclin D1 levels in all cell lines, but specifically knocking down cyclin D1 by siRNA failed to induce apoptosis in JeKo-1 or Granta 519 cells (Figs. 5C and 6C). These results are consistent with the work published by Klier and colleagues (21) using lentiviral short hairpin RNA–mediated knockdown of cyclin D1 in MCL cell lines. They reported retardation of growth without induction of apoptosis, and a small shift of S phase of cells to G1 phase. The compensatory induction of cyclin D2 expression or secondary genetic events may render cyclin D1 dispensable for maintaining MCL cell survival. Thus, these data indicate that the dysregulation of cyclin D1 may contribute to proliferation but does not offer a strong survival advantage. If so, cyclin D1 alone may not be a sufficient target for therapeutic designs.

In contrast to cyclin D1, expression of Mcl-1 was reported to be associated with blastoid/large-cell morphology and proliferative state in MCL (22). This variant of MCL, showing numerous medium-to-large blast-like cells, is associated with a more aggressive clinical course. It has been suggested that the high expression of Mcl-1 may contribute to proliferation but does not offer a strong survival advantage. If so, cyclin D1 alone may not be a sufficient target for therapeutic designs.

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The Granta 519 cell line was derived from the peripheral blood of a MCL patient with stage IV high-grade disease. However, this cell line is positive for EBV (24), which encodes viral proteins such as LMP-1, which has polytrophic functions to activate signal transduction and upregulate antiapoptotic proteins (24). Granta 519 cells overexpress the antiapoptotic protein Bcl-2 (25), which may be one of the consequences of EBV infection. Thus, Granta 519 represents a variant of MCL that has an alternative survival mechanism to sustain cell viability compared with JeKo-1 cells. Nevertheless, SNS-032 clearly inhibited Granta 519 proliferation (Fig. 1A) and clonogenic survival (Fig. 1C). Diminishing the cyclin D1 level may contribute to this effect, as knocking down cyclin D1 by siRNA led to significant reduction in clonogenicity (Fig. 6D), as did a short hairpin RNA approach (26). There was clear reduction of the cyclin D1 protein level (Fig. 3C) as well as reduced phosphorylation of Rb (Fig. 4B) after incubation with SNS-032. Furthermore, inhibition of Cdk2 activity in Granta 519 cells was evident from the reduced phosphorylation of Rb at serine 821 (Fig. 4B). Thus, the combined actions of transcriptional inhibition, decreasing cyclin D1 protein, and inhibition of proliferation through Cdk2 may cause the loss of clonogenic
survival in Granta 519 cells following SNS-032 exposure. Consistent with this, Cdk antagonists with inhibitory activity against both transcription and cell cycle, such as flavopiridol (27) and roscovitine (28), have shown induction of cell death in MCL cell lines (18, 29). Compared with flavopiridol and roscovitine, SNS-032 is more selective (4) and showed greater potency in the inhibition of transcription and induction of apoptosis in primary CLL cells (5).

It is known that both Mcl-1 and cyclin D1 proteins contain signals that program their rapid turnover (<1 h; refs. 30–32). Thus, even transient inhibition of translation could lead to a reduction of these protein levels. For instance, using a fusion toxin to specifically deliver a translational inhibitor gelonin to B cells, Lyu and colleagues (33) reported efficient inhibition of cell growth and induction of apoptosis in JeKo-1, Mino, and SP-53. Because of the labile feature of Mcl-1, gelonin may have acted similarly to SNS-032 to suppress Mcl-1 protein and induced apoptosis. A more direct approach could be to use an inhibitor of translation, such as homoharringtonine (34), which has been shown to reduce cyclin D1 (35) and Bcr-Abl levels (12). The mTORC1 inhibitor rapamycin and its derivatives have also shown effectiveness in MCL cell lines (36, 37), and clinical trials showed that these compounds have therapeutic benefit for relapsed MCL (38).

Our results showed that 0.3 μmol/L SNS-032 was the maximally effective concentration in all cell lines. This concentration was achieved at the 75 mg/m² dose level in the phase I clinical trial of SNS-032 in B-cell malignancies (6). Biomarker modulations, including inhibition of RNA pol II phosphorylation, marked reduction of Mcl-1 levels, and induction of apoptosis was observed in CLL samples taken from the patients during the clinical trial. Because only moderate clinical activities were observed in these patients, it was not possible to evaluate the relationship between pharmacodynamics and clinical response. In addition to SNS-032, other Cdk inhibitors with similar mechanisms of action such as roscovitine,
flavopiridol, and SCH 727965 are also under evaluation in clinical trials in B-cell malignancies (39, 40). Thus, this in vitro study provided a rationale for clinical evaluation of these Cdk inhibitors in MCL.

In conclusion, we investigated the action of Cdk inhibitor SNS-032 in a proliferating cell model in four MCL cell lines. Our results showed that SNS-032 has inhibitory activity on both transcription and cell cycle progression. Although cytotoxicity was shown in all lines, the mechanism of action was dependent on the biological context of individual cell lines. Thus, SNS-032 may be an active compound against different variants of MCL through distinct mechanisms of action.

Disclosure of Potential Conflicts of Interest

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