SNS-032 is a potent and selective CDK2, 7 and 9 inhibitor that drives apoptosis in the multiple myeloma cell line RPMI-8226

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Abstract

SNS-032 (SNS-312) is a potent and selective inhibitor of the cyclin-dependent kinases (CDKs) 2, 7 and 9 in normally in a phase of cell cycle progression and is used to treat solid and hematologic malignancies. SNS-032 works to inhibit cancer cells by blocking both the cell cycle and transcription. The cell cycle-dependent component of SNS-032 is known to drive the induction of apoptosis of CDK2 and 7 and 9 progression through SNS-032, acting within the Cdk complex, serves as a “master regulator” of CDK activity. Blockade of RNA pol IIdependent transcription via SNS-032 is known to drive the induction of CDK7 and CDK9. Transcriptional inhibition of all three CDKs results in the inhibition of RNA polymerase II-dependent transcription.

Background

SNS-032 is currently in a phase 1 trial for the b-myeloid malignances chronic lymphocytic leukemia, multiple myeloma (MM) and myeloma stem cells. SNS-032 is administered by a 15 minute IV loading dose followed by a 6 hr IV infusion. This dose regime was pharmacologically-derived, designed to achieve a median terminal half-life of 24 hours. This was observed to be sufficient to drive commitment to apoptosis as determined by annexin V/PI staining and PARP cleavage. Induction of apoptosis correlated with target phosphorylation of RNA pol II CTD under the same treatment conditions.

Methods

Cell line: The RPMI 8226 cell line (ATCC: CCL-185) was cultured in RPMI 1640 media (Cellgro) with 10% FBS.

Colony formation assay: Following treatment with a dilution series of SNS-032, cells were washed twice with fresh media, counted and plated (2000 cells/well) in triplicate to 6 well plates with 1 ml of media and re-fed with fresh media. Following 7 day incubation, colonies were stained with Hoechst 33342 and counted using an ArrayScan HCS device.

Array Scan: Cells were treated for 2hrs with a serial dilution of SNS-032. Fixed and normalized with 10% methanol in 2×PBS. Cells were stained using ApoBrdU Detection kit (Invitrogen) and counterstained with nuclear dye Hoechst 33342. Cells were evaluated in the Lower Right Quadrant: early apoptosis. Upper Left/Right Quadrants: late apoptosis/necrosis.

Western blot: Cell lysates (30ug) were analyzed on 15% Tris-Glycine NuPAGE gels and transferred to PVDF membranes. Membranes were blocked in 5% milk in TBS and probed with the following antibodies: CyclinD1 (Cell Signaling), pS2 (Cell Signaling), pS5 (Cell Signaling), MCL-1 (Santa Cruz), XIAP (Cell Signaling), PI (Cell Signaling), Bcl-2 (Cell Signaling), and Bax (Cell Signaling). Membranes were then incubated with HRP-conjugated secondary antibodies and visualized using chemiluminescence reagents (Amersham GE Healthcare). Relative band intensities were quantified using Kodak Molecular Imaging system.

RNA was isolated from treated cells using Ambion’s RNAqueous 4PCR kit (#AM1914) and cDNA synthesized using Applied Biosystem’s cDNA Reverse Transcription Kit (4374966). cDNA samples were subjected to RT-PCR analysis following standard methods using a TaqMan device and gene expression kits from Applied Biosystems. Primers were designed using the Primer Express software (Applied Biosystems). All samples were run in triplicate. RT-PCR results were analyzed using the 2^(-ΔΔCt) method (Livak and Schmittgen 2001). Statistical significance was determined using the Student’s T-test.

Results

Figure 4. Six hour treatment with SNS-032 downregulates transcripts coding D1 and D2, VEGF, and survival signaling proteins.

RT-PCR data following exposure to 300nM SNS-032 treated cells compared to DMSO control. All samples were normalized to 18s rRNA. This transcript is not modulated by inhibition of RNA polymerase II.

Figure 5. Six hr treatment with 500MHz SNS-032 inhibits CD94- and CD11c-mediated phosphorylation of RNA pol II CTD. Phosphorylation levels were assessed with antibodies to pS2 and pS5.

Phosphorylation levels in both figures are represented as a percentage of phospho/poly(A) relative to vehicle-treated cells.

Figure 6. Six hour treatment with SNS-032 induces apoptosis in RPMI-8226 cells as detected by Annexin V and TUNEL staining.

Cells were treated with SNS-032 or DMSO (control) for 6 hrs followed by compound washout and further incubation for a time of 24hr.

Annexin V / PI staining and FACs analysis to detect early apoptosis and cell death

Low Left Quadrant: normal cells

Low Right Quadrant: early apoptosis

Upper Right Quadrant: Late Phase (50%-90%) apoptosis

TUNEL staining and FACs analysis to detect apoptosis Fragmentation of DNA, Representative of late stage apoptosis.

HL: normal cells

H2: apoptosis cells

Summary & conclusions

• SNS-032 is a potent, selective inhibitor of CDKs 2, 7 and 9 that inhibits both cell cycle progression and transcription

• SNS-032 abrogates dysregulated proliferation as demonstrated by inhibition of colony formation by RPMI-8226 MM cells

• SNS-032 inhibits the phosphorylation of ser9 and ser2 of RNA Pol II CTD, consistent with mechanism-based inhibition of CDks 7 & 9

• Treatment of RPMI-8226 MM cells for 6 hrs is sufficient to induce apoptosis, as demonstrated by Annexin V and TUNEL staining as well as by PARP cleavage

• Exposure to SNS-032 for 6 hrs induces down-regulation of short half-life proteins and/or transcripts including cytokins 12 & D2, VEGF and survival factors Bcl-2, Bcl-xL and Mcl-1

• These data support the pharmacologically-derived 6 hr infusion regimen in the ongoing Phase 1 clinical trial of SNS-032 in b-lymphoid malignancies and demonstrate target modulation, anti-proliferative activity and induction of apoptosis. In addition, this study of SNS-032 is a true test of the hypothesis that targeted inhibition of CDKs 7 and 9 will be active in hematologic cancers by inducing apoptosis of malignant cells and disrupting tumor-stroma interaction requisite for maintaining these diseases.