



SNS-032 HAS POTENT ANTI-TUMOR ACTIVITY IN VIVO AGAINST HUMAN LEUKEMIA AND MULTIPLE MYELOMA XENOGRAPTS

Mamatha Reddy, Jennifer P. Arbitrario, Jeffrey Jones, Jeffrey A. Silverman, Anthony R. Howlett and Pietro Taverna
Sunesis Pharmaceuticals, Inc., South San Francisco, CA

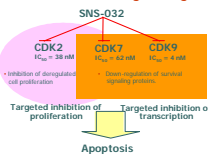
ABSTRACT # 258

SNS-032 (formerly known as BMS-387032) is a potent, selective inhibitor of cyclin dependent kinase (CDK) 2, 7 and 9 that inhibits both cell cycle progression and transcription and is in a Phase 1 clinical trial for the treatment of hematological malignancies.

We hypothesize that SNS-032 inhibits transcriptional initiation and elongation by blocking the phosphorylation of Ser5 and Ser2 of the C-terminal domain (CTD) of RNA pol II by CDK7 and CDK9, respectively. Short half-life (T1/2) transcripts and proteins are maximally affected by transient exposure to SNS-032. Down-regulation of short T1/2 survival signaling proteins by SNS-032 was recently demonstrated in a multiple myeloma cell line (RPMI-8226) (Conroy et al AACR 2007). Clinical evidence of SNS-032 target modulation was demonstrated ex vivo in peripheral blood mononuclear cells from treated cancer patients (Havatin et al. Haematologica 2007;92,suppl 1). In this study, we investigate the in vivo anti-tumor activity of SNS-032 and correlation with potential pharmacodynamic (PD) markers of activity in athymic mouse subcutaneous xenograft models of human acute leukemia (MV4-11 and HL-60) and multiple myeloma (RPMI-8226). Dosing was initiated when tumor volumes were on average at least 200 mm³. Tumor growth was monitored throughout the study and PD endpoints were analyzed by Western blot. Single IP administration of 30 or 15 mg/kg of SNS-032 induces decreased phosphorylation of both Ser2 and Ser5 of RNA Pol II CTD in tumor lysates from mice with MV4-11, HL-60 and RPMI-8226 xenografts, consistent with inhibition of CDKs 7 and 9. This occurs as early as 2 hours post dose and is sustained for at least 6 hours. In HL-60, the effects of SNS-032 on RNA Pol II phosphorylation correlate with down-regulation of the pro-survival protein MCL-1 at 30 mg/kg. PARP cleavage, indicative of apoptosis, is observed at both 15 and 30mg/kg dose levels. Administration of SNS-032 qd x5 at the MTD (30 mg/kg) results in 121% and 130 % tumor growth inhibition (TGI) in HL-60 and RPMI-8226 xenografts, respectively. Moreover, SNS-032 has potent anti-tumor activity against HL-60 xenografts using an intermittent q4d schedule at MTD and 1/2 MTD, with 119% and 86% TGI, respectively. When 30 mg/kg SNS-032 is administered qd x5 in the HL-60 xenograft model, complete responses are observed in 7 out of 10 animals on day 78; q4d administration of SNS-032 for 3 doses results in complete responses in 6 out of 10 animals on day 78. Animals remain tumor free for at least 78 days post-treatment. Similarly, 5/9 RPMI-8226 engrafted mice have complete responses and do not relapse 78 days following 5 daily doses of 30 mg/kg SNS-032. These results show that in models of hematologic malignancy, the in vivo activity of SNS-032 is associated with modulation of CDK7 and CDK9, modulation of short T1/2 survival signaling proteins such as MCL-1, induction of apoptosis and sustained tumor regression. Taken together, these studies support the therapeutic hypothesis being tested in an ongoing Phase 1 clinical study of SNS-032 in patients with chronic lymphocytic leukemia and multiple myeloma.

BACKGROUND

Therapeutic Hypothesis For SNS-032 Treatment Of Hematologic Malignancies

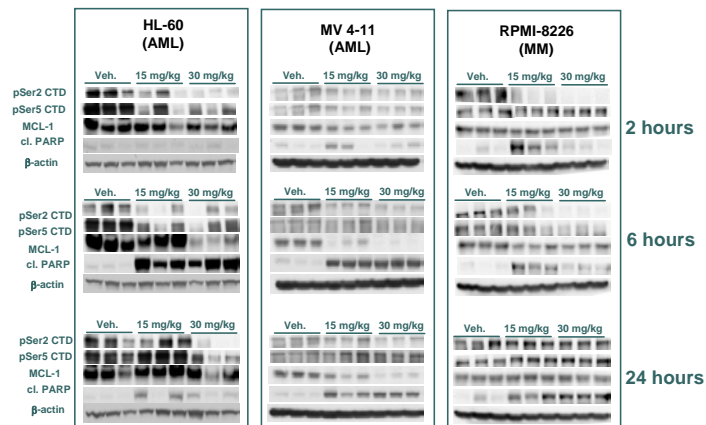


- We have previously shown in a MM cell line that SNS-032 activity correlated with inhibition of RNA pol II CTD phosphorylation and down regulation of short half-life survival proteins
- In this study we explore the relationship between SNS-032 mechanism of action and in vivo efficacy by evaluating potential bio-markers of CDK7 and CDK9 activity
- We also investigate the optimal dosing and schedule of SNS-032 in xenograft models of human leukemia and MM

SNS-032 induces potent inhibition of RNA pol II CTD phosphorylation and a sustained pro-apoptotic response in human xenografts

Experimental Design and Methods:

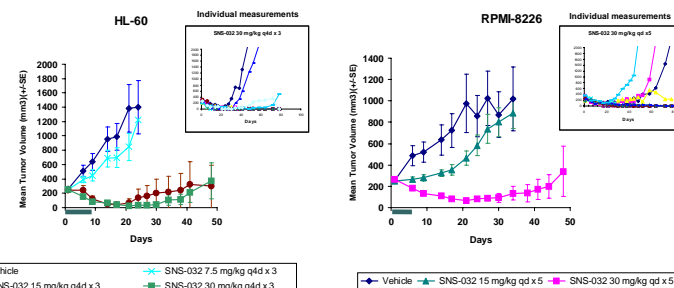
- Tumor bearing mice were dosed IP with a single injection of SNS-032
- Tumors were collected at 2, 6 and 24 hours post-dose
- 40 µg of total proteins were separated on 4-12%Tris-Glycine NuPAGE gels (Invitrogen)
- After transfer, nitrocellulose membranes were probed for RNA pol II pS2 CTD (Abcam 5095), RNA pol II pS5 CTD (Abcam 5131), MCL-1(BD Pharmingen 554103), cleaved PARP (Cell Signaling 9541), β-actin (Sigma A-2228).



Results:

- RNA pol II** is significantly modulated after single doses of 15 or 30 mg/kg SNS-032
 - pSer 2 CTD modulation appears greater than pSer 5 CTD modulation after SNS-032 treatment
 - Drug-induced modulation is already evident 6 hr after SNS-032 administration and sustained at least for 24 hr after 30 mg/kg single dose in HL-60 and MV 4-11
- MCL-1** protein levels are modulated differently in the 3 xenografts with MV 4-11>HL-60>RPMI-8226
 - MCL-1 modulation is sustained for 24 hr after 30 mg/kg SNS-032 in MV 4-11 and HL-60
- PARP cleavage**, indicative of apoptosis, is evident after a single 15 and 30 mg/kg SNS-032 dose
 - Increased levels of PARP cleavage are detected as early as 6 hours post-dose and sustained for at least 24 hours
 - PARP cleavage does not correlate with MCL-1 modulation in these 3 xenografts

SNS-032 causes regression of leukemia and multiple myeloma xenografts



- SNS-032 is well tolerated and highly efficacious in HL-60 and RPMI-8226 tumor bearing mice after intermittent or daily treatment schedules
- Multiple long term regressions of xenografts were observed in all the models investigated to date
 - 6/10 HL-60 tumor-bearing mice were still tumor free 78 days after administration of 3 intermittent (q4d) doses of 30 mg/kg SNS-032
 - 5/9 RPMI-8226 tumor-bearing mice were still tumor free 78 days after administration of 5 daily doses of 30 mg/kg SNS-032

All in vivo experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Sunesis

Summary and Conclusions

- SNS-032 is a selective inhibitor of CDKs 2,7 and 9 that shows potent activity against cellular and xenograft models of hematological malignancies
- SNS-032 inhibits in vivo the phosphorylation of Ser2 and Ser5 of RNA Pol II CTD, consistent with inhibition of CDKs 9 and 7
- pSer 2 CTD (CDK9) modulation appears greater than pSer 5 CTD (CDK7) modulation after SNS-032 treatment
- A sustained apoptotic response (indicated by PARP cleavage) is observed in xenografts of hematologic malignancy following treatment with SNS-032
- Potent anti-tumor activity following SNS-032 administration is demonstrated by multiple tumor regressions after intermittent, well tolerated treatment schedules
- Our study supports investigation of SNS-032 in a Phase 1 clinical study in patients with chronic lymphocytic leukemia and multiple myeloma <http://clinicaltrials.gov/ct/show/NCT00446342>