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.
  - pSer2 CTD modulation appears greater than pSer5 5 CTD modulation after SNS-032 treatment.
  - Drug-induced modulation is already evident 6 hr after SNS-032 administration and sustained for at least 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 xenografts.
  - 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 all 24 hours.
  - PARP cleavage does not correlate with MCL-1 modulation in these 3 xenografts.

**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 CDK7 and CDK9.
- PARP cleavage does not correlate with MCL-1 modulation in these 3 xenografts.

**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.

**Background**

- SNS-032 (formerly known as BNG-307032) is a potent, selective inhibitor of cyclin dependent kinase (CDK) 2, 7, and 9 that inhibits both cell cycle progression and transcription and 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, as validated by CDK7 and CDK9, respectively.
- Short half-life (TL) 2 transcripts and proteins are maximally affected by transient exposure to SNS-032.
- Down-regulation of short TL 2 specific signaling proteins by SNS-032 were recently demonstrated in a multiple myeloma cell line (RPMM-8226) (Corry et al AACR 2007).
- Clinical evidence of SNS-032 target modulation was demonstrated in vivo in peripheral blood mononuclear cells from treated cancer patients (Hawtin et al, Haematologica 2007;92(suppl 1)).
- In this study, we investigate in 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 (RPMM-8226).
- Dosing was initiated when tumor volumes were on average at least 200 mm3.

**RPMI-8226**

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**Summary and Conclusions:**
- 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 (qdx5) 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.**