



# SNS-595, A Novel S-phase Active Cytotoxic, Exhibits Potent *In Vitro* And *In Vivo* Activities, And Has The Potential For Treating Advanced Hematologic Malignancies

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## ABSTRACT #4726

SNS-595 is a novel naphthyridine analog that acts specifically during the S phase to induce rapid apoptosis of cells that are actively synthesizing DNA, resulting in cell cycle arrest in the G2 phase. SNS-595 is being developed for the treatment of both solid and hematologic malignancies. Current treatment of relapsed or refractory acute leukemias and advanced chronic myelogenous leukemia remains suboptimal. In patients with acute myelogenous leukemia (AML), failure to achieve a complete remission (CR) after induction chemotherapy or relapse occurring within the first year after diagnosis is associated with a poor prognosis. In patients with accelerated or blast-phase chronic myelogenous leukemia (CML) the response rates to intensive chemotherapy regimens are less than 30%, and in responding patients the median remission duration averages 4 to 6 months. SNS-595 has potent anti-proliferative effects on human leukemic cell lines *in vitro*.  $IC_{50}$  values, determined by MTT assay in four human promyelocytic, and acute lymphoblastic leukemia cell lines, range from 40 to 170 nM. Intravenous administration of SNS-595 to nu/nu mice with LM3-Jck hematologic xenograft tumors resulted in 97 to 99% tumor growth inhibition. All mice treated with SNS-595 had significant reductions in tumor volume, with a complete response rate of 67%. Similarly, SNS-595 also showed 98% tumor growth inhibition in mice bearing CCRF-CEM xenograft tumors.

The effect of IV administered SNS-595 on bone-marrow cellularity and circulating levels of leukocytes in CD-1 mice was measured after drug administration on days 0 and 4. Two days after the second administration of SNS-595, bone marrow isolated from femurs showed a dose-dependent reduction in cellularity. At 20 mg/kg, cellularity was reduced to 7.5%, while circulating neutrophils were reduced from a pre-dose level of  $1244 \pm 55$  cells/ $\mu$ L to a nadir of  $51 \pm 24$  cells/ $\mu$ L blood on day 8. Absolute neutrophil counts subsequently rebounded and soon returned to normal levels. Total WBCs also reached a nadir on day 8, but returned to normal levels. In conclusion, *in vitro* and *in vivo* data show that SNS-595 has potent anti-proliferative activity on human leukemic cell lines, inhibits tumor growth in hematologic xenografts, and reversibly ablates murine bone marrow cells. These data suggest that SNS-595 has the potential to demonstrate important clinical activity in patients with hematologic malignancies.

## BACKGROUND

SNS-595, a naphthyridine derivative, is a novel cell cycle targeted agent intended for the treatment of several tumor types. The cytotoxic activity of SNS-595 has been demonstrated in more than 20 different tumor cell lines, and antitumor activity has been observed in 11 human xenograft tumor models and 3 syngeneic models in mice. SNS-595 has a unique mechanism of action: it causes genotoxic signals through both p53 and p73 pathways during DNA synthesis, accompanied by a rapid apoptosis onset and an irreversible G2 arrest. These genotoxic signals are consistent with a DNA damage insult and response. SNS-595 distinguishes itself from other compounds with rapid checkpoint signaling and immediate cell death via apoptosis.

## METHODS

### MTT cell viability assay:

Cells were seeded in 96 wells plates at 3000 cells per well, and incubated for 16 hours. Compound dilutions were performed in DMSO from 10mM with 3 fold dilutions. Titrations were diluted 1:100 in media to achieve final compound concentrations. The 96 well plates were aspirated and compound dilutions in media were added (100 $\mu$ L/well). MTT analysis was carried out after 72 hours of incubation at 37 $^{\circ}$  C. Briefly, 20 $\mu$ L of MTT solution was added to each well. Cells were incubated at 37 $^{\circ}$  C for 1-2 hours. Cells were lysed with the addition of 100  $\mu$ L/well cell lysis buffer and MTT was solubilized overnight at 37 $^{\circ}$  C. Plates were read on a spectromax machine with an absorbance measurement at 570nm.  $IC_{50}$ 's were calculated using regression analysis within GraphPad Prism.

### Xenograft models:

LM3-Jck human malignant lymphoma tumor lobes (2-3mm square) were transplanted subcutaneously into nude mice. Tumors were allowed to grow to approximately 7-14 mm in diameter. Mice were pair-matched into no treatment, Irinotecan (100 mg/kg, IV, q4d x 3), Doxorubicin (12 mg/kg, IV, Single shot), Etoposide (12 mg/kg, IV, q1d x 5), and SNS-595 (25 and 20 mg/kg, IV, q7d x 5) treatment groups. Acceptable toxicity was defined as a mean group weight loss of 30% or less and not more than one toxic death among 6 treated animals. Anti-tumor activities of the drugs were assessed 21 days after the start of administration.

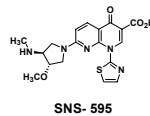
CCRF-CEM acute lymphoblastic leukemia tumor lobes of 2-3 mm square were transplanted subcutaneously into nude mice. Tumors were allowed to grow to approximately 8-20 mm in diameter. Mice were pair-matched into no treatment, Irinotecan (100 mg/kg, IV, q4d x 3), Doxorubicin (12 mg/kg, IV, q7d x 3), Etoposide (12 mg/kg, IV, q1d x 5), and SNS-595 (25 and 20 mg/kg, IV, q7d x 5) treatment groups. Acceptable toxicity was defined as a mean group weight loss of 30% or less and not more than one toxic death among 6 treated animals. Anti-tumor activities of the drugs were assessed 20 or 21 days after the start of administration.

### Bone Marrow / Cytology:

Female CD-1 mice were administered 5, 10, 15, or 20 mg/kg SNS-595 intravenously on Day 0 and Day 4. Blood was drawn on days 6, 8, and 12 post initial injection for hematological analysis. Femurs were extracted on day 6 fixed in Streck and H&E stained prior to bone marrow cellularity analysis.

## SNS-595 IS ACTIVE IN HEMATOLOGIC CELL MODELS

### SNS-595 SHOWS POTENT ANTI-PROLIFERATIVE ACTIVITY AGAINST HEMATOLOGIC CELL LINES

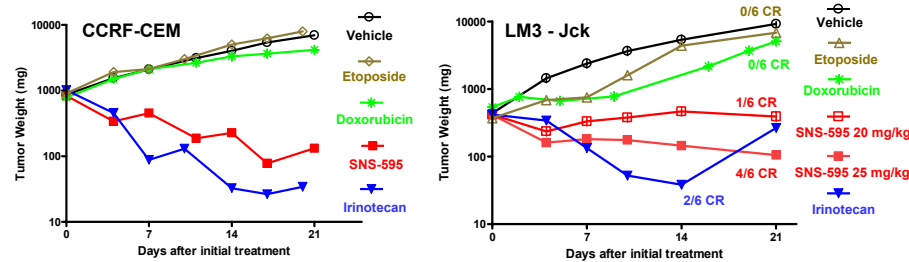


Cell Line	SNS-595	Etoposide	Doxorubicin	Irinotecan
	IC – 50 ng/mL			
HL-60	53	136	24	905
Jurkat	23	nd	nd	nd
CCRF-CEM*	18	nd	3	479
CEM/C2*	10	nd	17	44400

HL-60 – promyelocytic leukemia; Jurkat – T cell leukemia; CCRF-CEM – lymphoblastic leukemia; CEM/C2 – camptothecin resistant derivative of CCRF-CEM

### CCRF-CEM & LM3-Jck XENOGRAFT RESPONSE†

SNS-595 administered at 20 and 25 mg/kg shows strong antitumor activity with complete tumor regressions against LM3-Jck malignant lymphoma. Tumor inhibition rate (IR) of SNS-595 was similar to that of Irinotecan and superior to Etoposide and Doxorubicin in both the CCRF-CEM and LM3-Jck xenograft models.



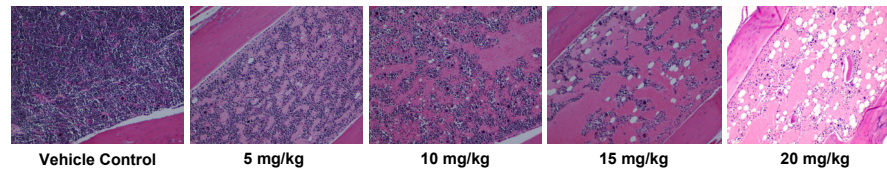
Treatment	Dose (mg/kg)	CCRF-CEM		LM3 - Jck	
		IR (%)	Survival Ratio	IR (%)	Survival Ratio
SNS-595 q7d X 3, IV	20	-	-	98.9*	6/6
	25	98.1*	6/6	95.8*	6/6
Irinotecan q4d X 3, IV	100	99.7*	5/6	97.7*	6/6
Doxorubicin q7d X 3, IV	12	50.3*	6/6	57.2*	6/6
Etoposide qd X 5, IV	12	28.3	6/6	3.0	6/6

# Nakano et al. 90th AACR Meeting Abstract # 767 † Kashimoto et al. 92nd AACR 2001 Abstract # 553

\* p<0.05

## BONE MARROW HISTOLOGY

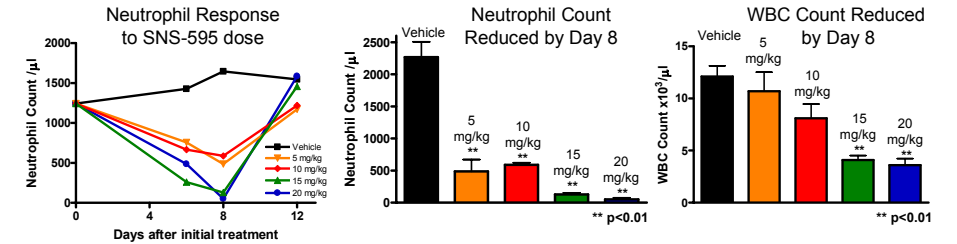
### SNS-595 DOSE DEPENDENT DECREASE IN THE HEMATOPOIETIC BONE MARROW CELLULARITY



Figures show cellularity in bone marrow 6 days post initial injection of SNS-595. SNS-595 was administered on day 0 and day 4. A reduction in cellularity was observed at all dose levels of SNS-595 relative to vehicle control. All images shown at 10x magnification.

## CYTOLOGY

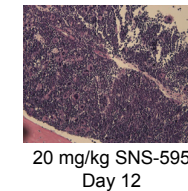
### SNS-595 SHOWS A PROFOUND REDUCTION IN PERIPHERAL BLOOD NEUTROPHIL COUNT



Neutrophil and total white blood cell counts were determined from blood samples on days 4, 6, 8, and 12 post initial injection. All SNS-595 dose groups demonstrated a significant decrease in peripheral neutrophils by day 8. Animals receiving 20 mg/kg injections of SNS-595 had less than 50 cells/ $\mu$ L on day 8.

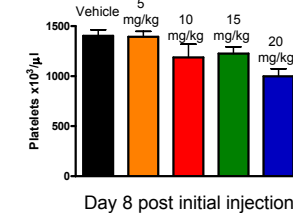
### BONE MARROW CELLULARITY AND PERIPHERAL BLOOD NEUTROPHIL ABLATION IS ACHIEVED AT SAFE DOSES OF SNS-595

#### Bone Marrow Rebounds



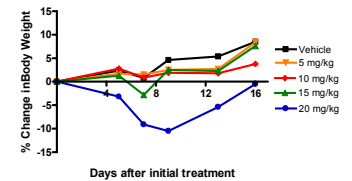
20 mg/kg SNS-595 Day 12

#### Minor Platelet Response to SNS-595 Dose



Day 8 post initial injection

#### Tolerable Body Weight Loss Post SNS-595 Injection



## SUMMARY & CONCLUSIONS

### Summary:

✓ SNS-595 is currently in a phase 1 clinical trial for advanced hematologic tumors and two Phase 2 studies for solid tumors.

✓ SNS-595 is active in hematologic cell line models

- Shows potent anti-proliferate activity against hematological tumor cell types
- Active in both the CCRF-CEM and LM-3 Jck xenograft models
- Induces complete regressions in the LM-3 Jck xenograft model

### Conclusions:

✓ SNS-595 has a profound effect on bone marrow and blood cellularity at safe doses

- Dose dependent loss in bone marrow cellularity
- SNS-595 significantly reduces blood neutrophil levels

– For additional SNS-595 poster presentations on *in vitro* combinations, mechanism of action, and clinical results see abstracts 2132, 2074, and 2913, respectively.