



SNS-595, A NOVEL CELL CYCLE INHIBITOR IN PHASE I CLINICAL TRIALS, CAUSES TUMOR REGRESSIONS, CELL-CYCLE ARREST, AND APOPTOSIS IN MURINE MODELS OF CANCER

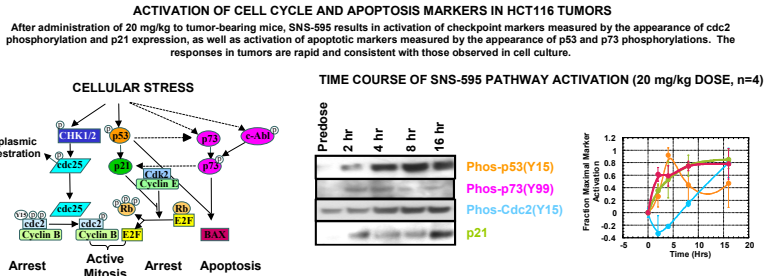
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ABSTRACT #2277

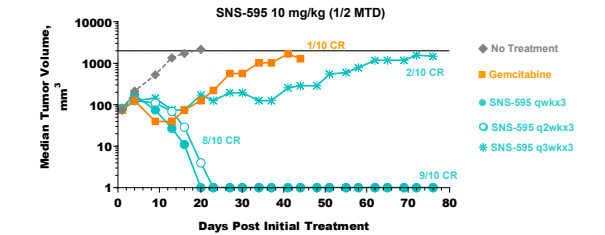
BIOLOGIC ACTIVITY OF SNS-595 IN HCT116 TUMORS

CURES IN COLO-26 SYNGENEIC MODEL WITH SPARSE DOSING

SNS-595 is a novel cell cycle modulator currently in clinical trials for the treatment of advanced solid malignancies. SNS-595 shows excellent antitumor activity in a broad spectrum of murine syngeneic and human xenograft tumor models, including drug-resistant models. Detailed cell cycle and pathway analyses in cell culture reveal that SNS-595 acts during S-phase and causes a significant S-phase lag associated with rapid appearance of checkpoint markers and sustained and irreversible arrest with 4N DNA content. This cell cycle arrest is rapidly followed by the onset of apoptosis, which is mediated through p53 independent and dependent mechanisms. To confirm the biological activities observed in cell culture in vivo, we analyzed tumor homogenates after single administration of SNS-595 to mice bearing advanced HCT-116 tumors. Within 24 hr of SNS-595 administration, modulations of pharmacodynamic markers consistent with G2 cell cycle arrest (cyclin B and cdc 2) and apoptosis (including p53, p21, and caspase-3) were detected. The pharmacodynamic effects in tumors correlated with tumor exposure to SNS-595 and contributed to significant growth delay of subcutaneously implanted HCT116 tumors when dosed on a weekly schedule. In addition to a weekly schedule (qwk x 3), less frequent schedules (q2wk x 3 and q3wk x 3) were studied in mice bearing subcutaneous Colo-26 tumors, a highly metastatic syngeneic colorectal tumor model known to be refractory to many of the major classes of cytotoxic drugs. SNS-595 proved to be highly active on all schedules with complete regressions and cures when dosed at the MTD. When dosed at 1/2 the MTD, the weekly and biweekly regimens still resulted in cures, whereas a reduction of the curative effect was apparent with the least frequent regimen. These studies with SNS-595 provide evidence that the strong tumor responses in animal models are mediated by biological activities consistent with those observed in cell culture.

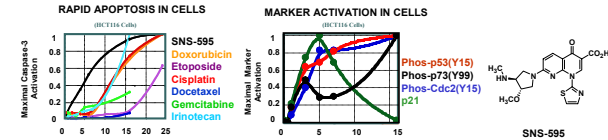


SNS-595 shows tumor regression and cures in the highly metastatic syngeneic Colo-26 tumor model known to be refractory to many of the major classes of cytotoxic drugs. SNS-595 is highly active on all schedules with complete regressions and cures when dosed at the MTD of 20 mg/kg. When dosed at 1/2 the MTD, the weekly and biweekly regimens still result in cures, whereas a reduction of the curative effect was apparent with the least frequent regimen.



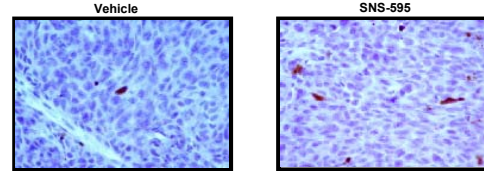
BACKGROUND

SNS-595, a naphthyridine derivative, is a novel cytotoxic 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 an S-phase lag, a rapid onset of apoptosis and an irreversible G2 arrest. SNS-595 distinguishes itself from other therapeutics with rapid checkpoint signaling leading to immediate cell death or regression (abstracts #2285 and #2293).

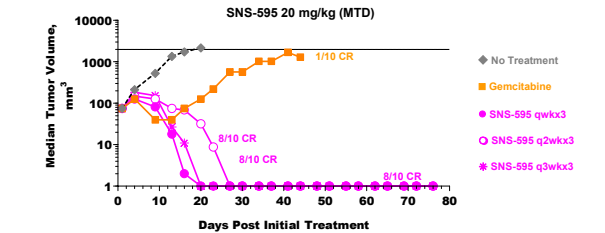
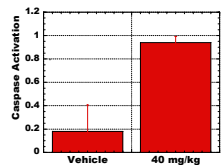


ACTIVATION OF CASPASE-3 IN HCT-116 TUMORS

Activation of Caspase-3 in a tumor sections. Tumors were removed 4 hr after administration of 20 mg/kg SNS-595 and stained with a Caspase-3 antibody.



Activation of Caspase-3 in tumor lysates. Tumors (n=3) were removed 6 hr after administration of 40 mg/kg SNS-595.



METHODS

All in vivo studies were performed in accordance with IACUC guidelines and in harmony with the Guide for Laboratory Animal Care and Use.

Pharmacodynamic Study: SNS-595 was administered intravenously to animals with established HCT116 tumors (300 mm³ in size). Tumor and blood (n=1 per time point) were collected at the indicated times post dose. A 50 mg tumor piece was homogenized with 5X buffer. SNS-595 levels in plasma and tumor homogenate were determined after protein precipitation by LC-ESI-MS/MS. The remainder of the tumor was ground into powder for Western or ELISA analysis. Tumor lysates were prepared and 10-20 µg total protein was run on 10% NuPage Bis-Tris Gel and then transferred to a Nitrocellulose membrane and probed using 1st and 2nd mAb (p21 Cellsignaling 2846, phos-p53(Y15) Cellsignaling 9284, phos-p73(Y99) Cellsignaling 4665). 50 µg total protein was used in a capture ELISA. Maxisorp Plate → anti-caspase-3 (BD 610322) → lysate → anti-cleaved caspase-3 (Cellsignaling 9661) → 2nd mAb-HRP (Chemcon)

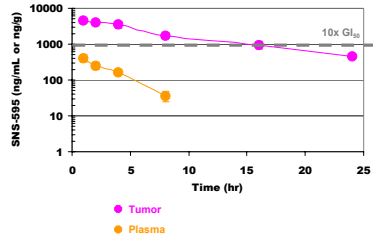
HCT-116 Tumor Model: HCT-116 colon carcinoma cells were implanted subcutaneously in the flank. Tumors were allowed to grow to approximately 100 mm³ in size. Mice were dosed with SNS-595 (10 mg/kg, IP, qwkx3) and SNS-595 (20 and 15 mg/kg, IV, qwkx3, q2wkx3, q3wkx3) treatment groups. Mice were weighed, first daily then twice a weekly and examined frequently for clinical signs of adverse effects. Acceptable toxicity was defined as a mean group weight loss of 20% or less and not more than one toxic death among 8 treated animals. Animals were euthanized when the tumor size reached 1500 mm³. Mean tumor volume was plotted until >25% of animals were lost per group.

Colo-26 Tumor Model: Colon carcinoma 26 cells were implanted subcutaneously in the flank. Tumors were allowed to grow to approximately 90 mm³ in size. Mice were pair-matched into no treatment, Gemzar (160 mg/kg, IP, q3wkx4), and SNS-595 (20 and 15 mg/kg, IV, qwkx3, q2wkx3, q3wkx3) treatment groups. Mice were weighed, first daily then twice a weekly and examined frequently for clinical signs of adverse effects. Acceptable toxicity was defined as a mean group weight loss of 20% or less and not more than one toxic death among 10 treated animals. Animals were euthanized when the tumor size reached 2000 mm³. Mean tumor volume was plotted until >20% of animals were lost per group.

We would like to acknowledge Piedmont Research Center for their contributions to the HCT-116 and Colo-26 tumor models.

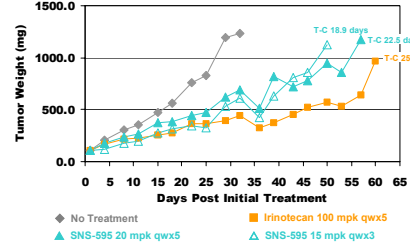
TUMOR AND PLASMA LEVELS AFTER 20 mg/kg IV DOSE

SNS-595 shows good tumor/plasma ratios and tumor concentrations above 10x the cellular GI₅₀ (2560 nM or 1000 ng/mL) for 16 hr.



HCT116 XENOGRAFT RESPONSE

SNS-595 shows tumor growth inhibition in HCT-116 xenograft model when dosed IV at or below the MTD of 20 mg/kg on a weekly dosing regimen.



CONCLUSIONS

- SNS-595 shows excellent distribution to tumor tissue resulting in tumor to plasma ratios of >10 in the tumor models tested
- SNS-595 shows biological activities consistent with those observed in cell culture
 - Activation of cell cycle markers in HCT-116 tumors, with maximum activation 2 to 16 hr post dose
 - Activation of apoptosis markers in HCT-116 tumors, with maximum activation 4 hr post dose
 - Stress signals observed in vitro lead to in vivo activity
- Preclinically, SNS-595 retains curative effects in the highly metastatic syngeneic Colo-26 tumor model, even when dosed every three weeks