



SNS-595 Has Synergistic Activity *In Vitro* With DNA Damaging Agents And Antimetabolites

SUNESIS

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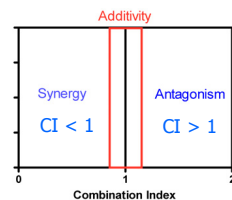
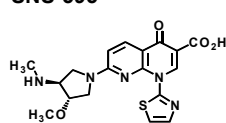
ABSTRACT

The ability to dose new antitumor agents in combination with established drugs is of key importance to developing new therapies. SNS-595 is a novel cytotoxic agent, currently in phase 2 studies, that rapidly activates apoptosis during S phase and has demonstrated broad antitumor activity in animal models. SNS-595 was added in combination with a panel of cytotoxic agents for 72 hours in the colon carcinoma cell line HCT116, the lung carcinoma line NCI-H460 and the ovarian carcinoma matched lines SKOV3 and SKOV3 (p53+/-). Cytotoxicity was determined by an MTT assay and the data was analyzed with CalcuSyn V2 (Biosoft) and represented as combination indices (CI). SNS-595 was found to be synergistic (CI < 0.85) when treated contemporaneously with the DNA damage agents etoposide, daunomycin, actinomycin D, mitomycin C, cisplatin, and carboplatin and with the antimetabolites pemetrexed, methotrexate, ara-C and 5-fluorouracil. Synergy was also demonstrated with the DNA-PK inhibitor wortmannin and the HSP90 inhibitor geldanamycin. SNS-595 was antagonistic when added to cells contemporaneously with the microtubule inhibitor docetaxel and the antimetabolite gemcitabine. However, synergy was achieved when cells were treated with docetaxel or gemcitabine prior to treatment with SNS-595. In most cases, cellular p53 status did not have a significant impact on the results. In conclusion, combining SNS-595 with DNA damaging agents, antimetabolites, DNA-PK inhibitors, microtubule inhibitors or HSP90 inhibitors could provide effective new treatments for patients suffering from various types of cancer.

BACKGROUND

SNS-595 is a novel cell-cycle active agent that causes double-strand breaks during S phase that are solely repaired through a DNA-PK-dependent pathway (Abstract # 2074). This mechanism suggests that SNS-595 could interact in combination favorably with other S-phase cytotoxics that may utilize different mechanisms of damage signaling and repair, such as DNA damaging agents and antimetabolites. Furthermore, drugs affecting NHEJ signaling and apoptosis, such as DNA-PK and Hsp90 inhibitors may also combine effectively with SNS-595. *In vitro* combination studies were undertaken with anticancer agents with varied mechanism of action in order to select agents most likely to show synergy *in vivo*. We evaluated combinations of SNS-595 with other anticancer agents using the median-effect method, which quantifies the interaction between two compounds by a term called the Combination Index (CI). When two compounds are combined, the effect can be the sum of the individual activities (additive) or the effect can be greater or less than additive (synergistic or antagonistic, respectively). The CIs for all interactions were calculated using a software program for Dose Effect Analysis called CalcuSyn V2 (Biosoft). CIs of 0.85-1.2 were considered to be additive, CIs > 1.2 antagonistic and CIs < 0.85 synergistic.

SNS-595



METHODS

Cell lines and Cell Culture: HCT116 and NCI-H460 cell lines were obtained from ATCC. SKOV3(p53-/-) and SKOV3(p53+/+) were generous gifts from the lab of Dr. George Stark of the Lerner Institute of the Cleveland Clinic. All cell lines were cultured in RPMI media supplemented with 10% FBS 1% Sodium Bicarbonate solution and 1% Antibiotic Solution (Cellgro).

MTT assay: Cells were plated at 4000 cells per well (except SKOV3 (p53-/-) which were plated at 8000 cells per well) in a 96 well plate, incubated for 24 hours and then treated with compound. Compound treatment lasted 72 hours. Cells were then incubated with 5% MTT for 1-2 hours, and lysed. MTT was colorimetrically read at 570nm. The fraction of dead cells was determined by

$$\text{Fraction of Dead cells} = 1 - \frac{[\text{Abs of sample well} - \text{Avg}(\text{Abs of no cell control})]}{[\text{Avg}(\text{Abs of DMSO only control}) - \text{Avg}(\text{Abs of no cell control})]}$$

Scheduling studies: When compounds were dosed with a schedule that included a washout, cells were washed with 100ul of fresh warm media for 30 minutes, followed by another wash after 90 minutes.

Statistical Analysis: The data (Fraction of Dead cells) was analyzed using CalcuSyn V2 (Biosoft) and is herein represented as the value of the Combination Index at Fraction affected (F_a) = 0.5. All data is shown with error bars indicating the 95% confidence intervals of the mean value.

SNS-595 IS SYNERGISTIC/ADDITIVE WITH VARIOUS AGENTS WHEN CO-DOSED

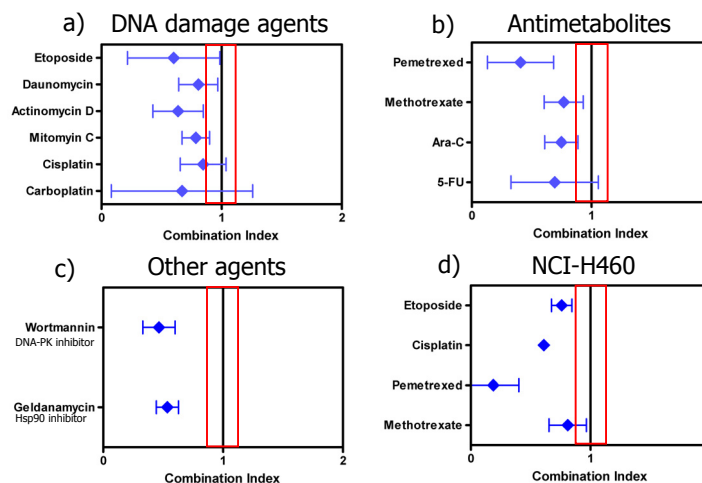
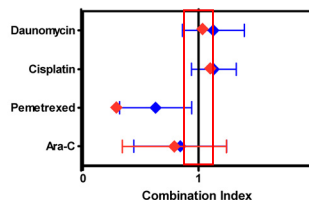


Figure 1. SNS-595 dosed simultaneously with various cytotoxics in HCT116 colon carcinoma cell line (a,b and c) and H460 lung cancer cell line (d) showed significantly synergistic or at least additive combination indices.

P53 STATUS DOES NOT AFFECT SNS-595 COMBINATION INDICES



Blue:SKOV3 (p53+/+)

Red:SKOV3 (p53-/-)

Figure 2. SNS-595 dosed simultaneously with a selection of DNA damaging agents and antimetabolites showed no significant change in the combination index between SKOV3 ovarian cancer cell line +/- p53 expression

Abstract Number: 2074
Presentation Title: SNS-595 causes selective double strand breaks during S-phase which are solely repaired through non-homologous end joining
Presentation Start/End Time: Monday, Apr 03, 2006, 8:00 AM - 12:00 PM
Abstract Number: 4726
Presentation Title: SNS-595, a novel S-phase active cytotoxic, exhibits potent *in vitro* and *in vivo* activities, and has the potential for treating advanced hematological malignancies.
Presentation Start/End Time: Tuesday, Apr 04, 2006, 1:00 PM - 5:00 PM
Abstract Number: 2913
Presentation Title: A phase 1 trial of weekly SNS-595 in patients (PTS) with refractory cancer
Presentation Start/End Time: Monday, Apr 03, 2006, 1:00 PM - 5:00 PM

ANTAGONISM WITH SNS-595 CAN BE ALLEVIATED WITH DOSE SCHEDULING

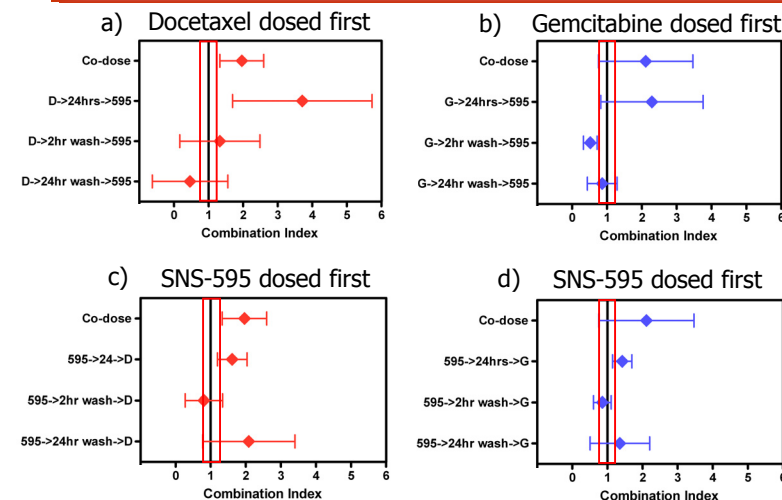


Figure 3. SNS-595 was dosed with docetaxel (a,c) and gemcitabine (b,d) simultaneously, with a 24hr delay and with a 2hr and 24 hr washout in HCT116 colon carcinoma cells. The most likely reason for schedule dependence of these combinations is that SNS-595 is an S-phase active cytotoxic and gemcitabine and docetaxel cause potent G1 and M phase arrests respectively.

SUMMARY & CONCLUSIONS

Summary:

- SNS-595 is a novel cell cycle active drug with a unique mechanism of action that shows broad activity in preclinical models
- SNS-595 can be effectively combined with a wide variety of other agents *in vitro*
 - Demonstrated synergy with other DNA damaging drugs and antimetabolites
 - Demonstrated synergy with DNA-PK inhibitors and Hsp90 inhibitors that affect NHEJ repair and apoptosis signaling
 - Modulators that abrogate the G2 checkpoint do not synergize with SNS-595 (Abstract # 2074).
 - Antagonism between SNS-595 and potent cell cycle cytotoxics that do not work in S-phase can be alleviated through dose scheduling
 - p53 status does not affect the combination indices between SNS-595 and other agents

Conclusions:

- These data suggest that SNS-595 may be effectively combined with a wide variety of cytotoxic drugs, chemotherapeutic modulators and novel agents that are active in S-phase or regulate NHEJ repair or apoptosis signaling.