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

Jasmin Wright, Jennifer Hyde, Jeffrey A. Silverman, Duncan H. Walker, Michelle R. Arkin
Sunesis Pharmaceuticals Inc, S San Francisco, CA

SNS-595 is a novel cell cycle active agent that causes double-strand breaks during S phase that are 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 of each agent or can be greater than the sum of the two effects (synergistic, 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 IS SYNERGISTIC/ADDITIVE WITH VARIOUS AGENTS WHEN CO-DOSED

METHODS

Cell lines and Cell Culture: HCT116 and NCI-H460 cells were obtained from ATCC. SKOV3(p53-/-) and SKOV3(p53+/+) were obtained from Dr. G. Daniel Stehlin at the University of California, San Francisco. All cell lines were cultured in RPMI media supplemented with 1% FBS, 1% Sodium Bicarbonate solution and 1% Antibiotic Solution (Cellgro). 96 well plate, incubated for 24 hours and then treated with compound. Compound treatment lasted 72 hours. Cells were then washed with 2% DTT for 1/2 hour, and treated with 5% MTT for 1-2 hours. The fraction of dead cells was determined by

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

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 and p53 expression.

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
- Antagonism between SNS-595 and potent cell cycle cytotoxics that do not work in S-phase can be alleviated through dose scheduling
- SNS-595 status does not affect SNS-595 combination indices

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