SNS-595, currently in phase I trials, is a novel cell cycle modulator with potent activity against various tumor models. SNS-595 treated cells have been shown to undergo rapid and cell-cycle-dependent apoptosis. Further characterization of the kinetics and mechanism of apoptosis in SNS-595-treated cells was conducted and compared to the activities of eight clinically relevant cytostatics (cisplatin, docetaxel, eloposide, gemcitabine, doxorubicin, vinorelbine, bleomycin, and mitomycin C). The relationship between the cell cycle and apoptosis was studied in both asynchronous and synchronized cell populations using several markers of p53-dependent and independent pathways (including p53, p73, c-Abl, and p21). In an asynchronous cell population, SNS-595 caused half maximal caspase-3 activation within 5 hrs of exposure; two times faster than the other cytostatics studied. Cells synchronized at G0/G1 and treated with SNS-595 displayed a steep increase in caspase-3 activation as the cells enter S phase. SNS-595 did not activate caspase-3 during M or G1 phases of the cell cycle, nor was caspase-3 activated in non-cycling cells. These results are consistent with cell cycle analysis indicating an S-phase lag, S-phase checkpoint activation, and G2 arrest following SNS-595 treatment. Analysis of the signaling pathways stimulated by SNS-595 indicates that apoptosis is stimulated through p53-dependent and independent mechanisms. In contrast to comparator compounds, SNS-595 stimulates p73 expression and caspase-3 activation rapidly (within 30 minutes) after p53 phosphorylation. Thus, SNS-595 stimulates the apoptotic cascade and subsequent cell death only when dosed during DNA synthesis; apoptosis follows stimulation of the p53, p73 and cell cycle checkpoint pathways. These distinctive cell cycle and apoptotic effects of SNS-595 will lead to further insight into the mechanism of action of this potent and novel cytotoxic compound.

**SNS-595: NOVEL S-PHASE CYTOTOXIC**

SNS-595 Treatment leads to rapid apoptosis in multiple cell lines, independent of p53 status. In HCT116 cells, SNS-595 induces half maximal Caspase-3 activation within 5 hrs of treatment, 2-10X faster than other chemotherapies studied.

**Dose Dependent Cellular Response**

SNS-595 shows steep activation of p53 dependent and independent pathways as the cells enter S phase. This initial stimulus is followed rapidly by Caspase-3 activation and cell death. These markers are not activated if SNS-595 is dosed in either a non-cycling cell population, or cells that are in G0, M or G1 phases of the cell cycle, no matter what the incubation time (data not shown and abstract #2293).

**Summary**

SNS-595: A Novel S-phase active agent

- Rapid apoptotic response in multiple cell lines (independent of p53 status)
- Novel response profile with rapid checkpoint signaling leading to immediate cell death or permanent arrest in the tested cell lines
- Activation of p53 dependent and independent pathways including a p73 response

**Conclusions**

SNS-595 is a novel cytotoxic whose cellular activities are independent of p53 status. The distinctive cell-cycle profile of SNS-595 - including S-phase specificity, activation of p73, and rapid apoptosis - signifies a novel mechanism of action compared to current therapeutics. Based on these results, different resistance profiles as well as novel drug combinations are being investigated.