Background: SNS-314 is a selective Aurora kinase inhibitor with potent preclinical anti-tumor activity in combination with standard chemotherapeutics and synergy with microtubule targeted agents. SNS-314 demonstrates potent single agent activity and demonstrates significant in vivo anti-tumor activity in a wide range of tumor xenograft models. Of importance, SNS-314 shows remarkable tumor growth inhibition using an intermittent schedule which provides potential for combining SNS-314 with other targeted and conventional anti-cancer therapeutics. To select combinations for testing in vivo, in vitro studies have been undertaken with anti-cancer agents with varied mechanisms of action.

Results: SNS-314 shows favorable toxicity in combination with commonly used anti-cancer agents. Sequential administration of SNS-314 with chemotherapeutic compounds showed additive anti-proliferative effects with carboplatin, gemcitabine, 5-fluorouracil (5-FU), and the active metabolite of irinotecan, SN38. Statistically significant synergy was observed in cells with sequential administration of SNS-314 followed by low doses of gemcitabine, or high doses of docetaxel or vincristine. The most profound anti-proliferative effects were observed with SNS-314 and agents that disrupt microtubule dynamics such as docetaxel, vincristine, and nocodazole.

Conclusions: SNS-314, a selective Aurora kinase inhibitor, demonstrates significant synergy in colorectal carcinoma cells with vincristine and docetaxel, and additive activity with all combinations tested. The in vivo synergy observed between SNS-314 and agents that target the mitotic spindle, and the potential for intermittent dosing in combination with the mechanism of action of an Aurora kinase inhibitor that bypasses an anti-mitotic spindle checkpoint resulting in mitotic catastrophe and cell death. SNS-314, a novel targeted Aurora kinase inhibitor, shows promise for rationally informed chemotherapeutic combinations for the treatment of human malignancies.

Methods

Cell treatment: HCT116 cells transfected with p53 RNAi or a control vector were used in a 10x EC50 concentration with SNS-314 (42.5 mg/kg, PO), Docetaxel (240 mg/kg, IV), Gemcitabine (10 nM, PO), and 5-FU (0.5 mg/kg, IV) for 24 hr. Tumor xenograft models were used to establish dose response curves for SNS-314 and are shown in the Supplementary Information section.

Combination index determination

A combination index compares the concentration of compounds administered in combination required for a given fractional effect to the concentration of single agent compound required to give the same fractional effect. In the approaches for fractional effect is the EC50 of Compound 1 and Compound 2, and the combination index (CI) is calculated as follows:

\[ CI = \frac{EC50_{1} + EC50_{2}}{EC50_{1} + EC50_{2}} \]

The equation above represents the theoretical additive response for two mutually exclusive drugs, and takes into consideration the ratios at which the two compounds are administered. When CI = 1, then drugs are additive. When CI < 1, less compound is required for a given fractional effect, and the combination is synergistic. When CI > 1, more compound is required, and the combination is antagonistic. The process by which CI were determined in the application is described in the figure below which illustrates hypothetical outcomes for interactions of two equipotent drugs (10 nM EC50).

Figure 1. Examples for the interaction of equipotent drugs and corresponding dose-responses. A, EC50 s are generated for both single agent and combinations. B, CI are calculated for mutually exclusive drugs, and taken into consideration the ratios at which the two compounds are administered. C, The Mann Whitney test was used to calculate a p-value and determine statistical significance from the additive internal control.

Figure 2. Results for combination studies conducted under three schedules. HCS cell count proliferation assay data tabulated from A and two additional studies. Dose schedules show the biweekly schedule (shown in A). Sequential, panel first, then treatment with SNS-314.

Figure 3. SNS-314 shows synergy with microtubule targeted agents.

Figure 4. Combination treated cells display evidence of endoreduplication and mitotic catastrophe. HCT116 cells, Cell Titer Blue proliferation assay (Promega).

Figure 5. SNS-314 shows potent docetaxel-based anti-tumor activity in vivo.

Figure 6. Sequential SNS-314-DTX dosing results in significant anti-tumor activity. Effects are observed at doses and schedules not efficacious as single agents in HCT116 xenografts. The plasma exposure (DTX > SNS-314, 24 hr separation) is not efficacious. Combination is not associated with additive toxicity compared to single agent treatments.

Summary and Conclusions

- The cytotoxic activity of SNS-314 is additive in vitro when administered in combination with standard chemotherapeutic compounds. Conditional synergies were seen in vitro for SNS-314 combined with gemcitabine, docetaxel, and vincristine.
- Most profound in vitro effects were observed when SNS-314 was followed by agents that target the mitotic spindle in dividing cells.
- As predicted in vitro, SNS-314 in combination with docetaxel results in significant anti-tumor activity at doses and schedules where neither compound showed single-agent activity in HCT116 xenografts.
- Preclinical studies further support development of SNS-314 with multiple potential dosing schedules and in combination with standard chemotherapies.
- SNS-314, a highly selective and potent pan Aurora kinase inhibitor is enrolling patients in a phase 1 dose-escalation study designed to assess safety and tolerability in patients with advanced solid malignancies.