

# SNS-314, a selective Aurora kinase inhibitor with potent, pre-clinical anti-tumor activity shows broad therapeutic potential in combination with standard chemotherapeutics and synergy with microtubule targeted agents

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## Abstract (Updated)

**Background:** SNS-314, a selective small-molecule inhibitor of Aurora kinases, has entered a phase 1 clinical trial for the treatment of patients with advanced solid cancers. Aurora kinases play critical roles during mitosis and cytokinesis. SNS-314 demonstrates significant *in vivo* 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* combination studies have been undertaken with anti-cancer agents with varied mechanisms of action.

**Results:** SNS-314 showed additive effects 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, daunomycin, 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 compounds tested. The *in vitro* synergies observed between SNS-314 and agents that target the mitotic spindle, and the potentiation seen with docetaxel *in vivo* are consistent with the mechanism of action of an Aurora kinase inhibitor that bypasses an activated 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 cultured in DMEM, 10% FBS, and 1x antibiotic/antimycotic. Cells were plated in growth medium in 384-well plates. Cells were treated to assess the effects of p53 status, drug dose ratios, and dose schedules. A dilution series of SNS-314 combined with a dilution series of various cytotoxics: gemcitabine (Gem), 5-FU, docetaxel (DTX), vincristine (VIN), carboplatin (Carbo), SN38, daunomycin (Dauno), cisplatin (Cis), nocodazole (NOC), or SNS-314 (internal additive control) was applied to cells. The three dose ratios tested were (314/Panel), high/high, low/high, and high/low, where the "high" compound dose response is generated starting at 10x EC50 and "low" compound is 1x EC50. Dose schedules were tested by combining compounds as a cosode, or sequential washout dose starting with either SNS-314 or a panel compound. All procedures were performed by a Tecan robotic platform.

**Cell count assay:** After overnight growth, cells were treated with compound for a total of 72 hours and incubated at 37°C, 5% CO<sub>2</sub>. Cells were fixed in formaldehyde and stained with Hoechst 33342. HCS images were captured and data analyzed according to the Target Activation application on the ArrayScan VTI instrument (Cellomics, Inc.).

**Proliferation assay:** Cells were plated and treated as described in the cell count assay with the exception of an extended incubation period of 6 days. A Cell Titer Blue (Promega) method was applied according to the manufacturer's instructions.

**In vivo anti-tumor activity:** Mice (nu/nu female) were SQ implanted with HCT116 colorectal carcinoma cells in the right hind flank with 200 µl of a 2.5 x 10<sup>7</sup> cell/mL suspension (1:1 DPBS with cells:Matrigel). When tumors reached an average volume of 200 mm<sup>3</sup>, mice were randomized into groups and treated IP with vehicle, SNS-314, or docetaxel (Taxotere®). All *in vivo* experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of Sunesis Pharmaceuticals, Inc., and in accordance with local state and Federal regulations. In combination, SNS-314 and docetaxel administration was separated by 24 hours.

## Combination index determination

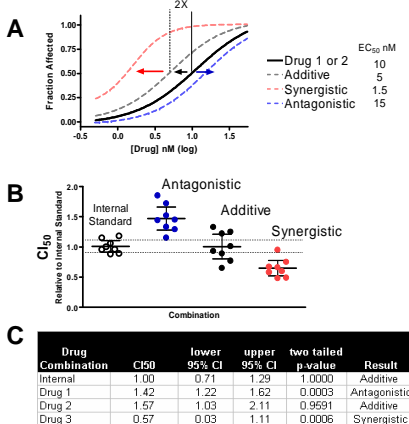
A combination index compares the concentration of compounds administered in combination required for a given fractional effect to the concentration of single agent compound required to give the same fractional effect. In this application, the fractional effect is the EC<sub>50</sub>.

$$CI_{50} = \frac{D_1}{D_{1,EC50}} + \frac{D_2}{D_{2,EC50}} = 1 \text{ Additive}$$

$$CI_{50} = \frac{D_1}{D_{1,EC50}} + \frac{D_2}{D_{2,EC50}} = 2 \text{ Antagonistic}$$

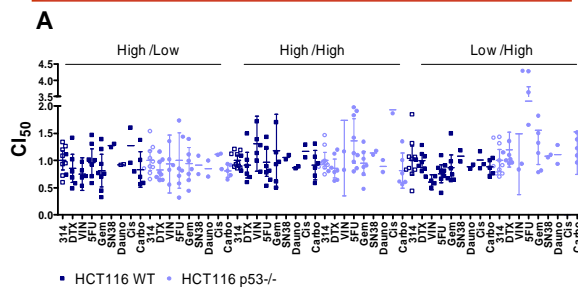
$$CI_{50} = \frac{D_1}{D_{1,EC50}} + \frac{D_2}{D_{2,EC50}} = 0.3 \text{ Synergistic}$$

The equation above represents the theoretical additive response for two mutually exclusive drugs, and takes into consideration the ratio at which the two compounds are administered. When CI<sub>50</sub> = 1 then drugs are additive. When CI<sub>50</sub> < 1, less compound is required for a given fractional effect, and the combination is synergistic. When CI<sub>50</sub> > 1, more compound is required, and the combination is antagonistic. The process by which CI<sub>50</sub>s were determined in this application is described in the figures below which illustrate hypothetical outcomes for interactions of equipotent drugs (10 nM EC<sub>50</sub>).



**Figure 1. Examples for the interaction of equipotent drugs as determined by corresponding dose-responses.** A. EC<sub>50</sub>s are generated for both single agent and combinations. B. CI<sub>50</sub>s are calculated for drug administered with itself, or in combination. Data from independent experiments are plotted with 95% confidence intervals. C. The Mann Whitney test was used to calculate a p-value and determine statistical significance from the additive internal control.

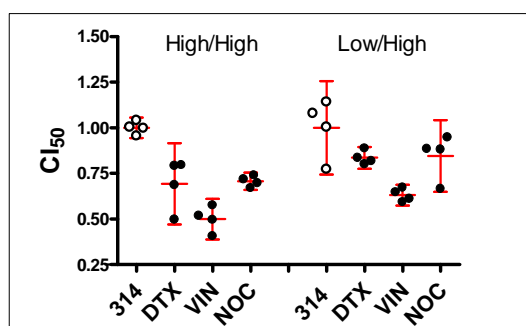
## Combining SNS-314 with common chemotherapeutics



Schedule	p53 RNAi	SNS-314		Panel		SNS-314		Panel	
		High	Low	High	Low	High	Low	High	Low
i. Cosode	SNS-314	5FU	Antagonistic	Antagonistic	Antagonistic	Additive	Additive	Additive	Additive
	Panel Drug	Carbo	Antagonistic	Antagonistic	Additive	Additive	Antagonistic	Antagonistic	
	Gem	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Additive	Additive		
	Dauno	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Additive	Additive		
	SN38	Additive	Antagonistic	Additive	Antagonistic	Additive	Additive		
	DTX	Antagonistic	Antagonistic	Additive	Antagonistic	Additive	Additive		
ii. Sequential, SNS-314 first	SNS-314	5FU	Additive	Additive	Additive	Additive	Additive	Additive	Synergistic
	Panel Drug	Carbo	Additive	Additive	Additive	Additive	Additive	Additive	
	Gem	Additive	Additive	Additive	Additive	Additive	Additive	Additive	
	Dauno	Additive	Additive	Additive	Additive	Additive	Additive	Additive	
	SN38	Additive	Additive	Additive	Additive	Additive	Additive	Additive	
	DTX	Additive	Additive	Additive	Additive	Additive	Additive	Additive	
iii. Sequential, panel first	SNS-314	5FU	Additive	Additive	Additive	Additive	Additive	Additive	Additive
	Panel Drug	Carbo	Additive	Additive	Additive	Additive	Additive	Additive	Antagonistic
	Gem	Additive	Additive	Additive	Additive	Additive	Additive	Additive	Antagonistic
	Dauno	Additive	Additive	Additive	Additive	Additive	Additive	Additive	Antagonistic
	SN38	Additive	Additive	Additive	Additive	Additive	Additive	Additive	Antagonistic
	DTX	Additive	Additive	Additive	Additive	Additive	Additive	Additive	Antagonistic

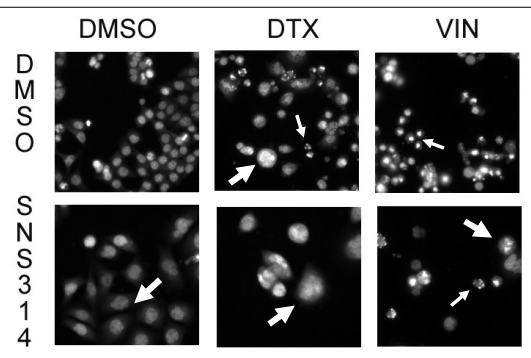
**Figure 2. Results for combination studies conducted under three schedules.** HCS cell count proliferation assay data tabulated from A and two additional studies. Dose schedules shown in B, i. Co-administration ii. Sequential SNS-314 first (shown in A), iii. Sequential, panel first, then treatment with SNS-314.

## SNS-314 shows synergy with microtubule targeted agents



314	Panel	High				Low				Result
		Mean	lower 95% CI	upper 95% CI	p value	Mean	lower 95% CI	upper 95% CI	p value	
314	1.00	0.94	1.06		1.00	0.74	1.26		Additive	
DTX	0.69	0.47	0.92	0.0286	0.84	0.78	0.90	0.343	Additive	
VIN	0.50	0.39	0.61	0.0286	0.63	0.57	0.69	0.029	Synergistic	
NOC	0.71	0.66	0.75	0.0286	0.85	0.85	1.04	0.200	Additive	

**Figure 3. Combination of SNS-314 with spindle toxins results in synergistic inhibition of cell growth.** Sequence is SNS-314, washout, docetaxel (DTX), vincristine (VIN), nocodazole (NOC). HCT116 cells, Cell Titer Blue proliferation assay (Promega).

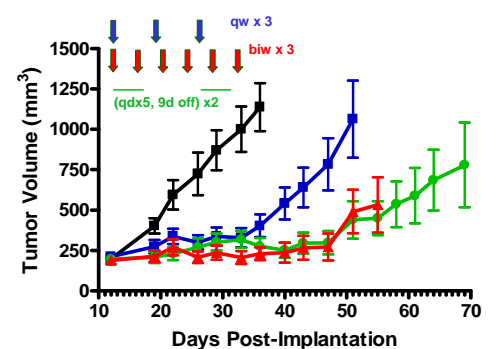


**Figure 4. Combination treated cells display evidence of endoreduplication and mitotic catastrophe.** HCT116 DNA morphologies reveal polyploidy (large arrows) and condensed or fragmented chromatin (thin arrows).

## 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 support further 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.

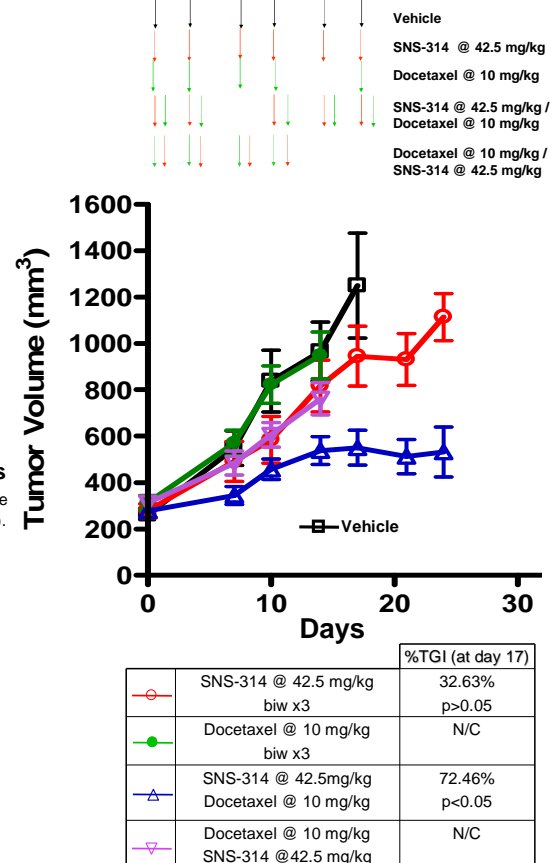
## SNS-314 demonstrates potent single agent activity



Dose (mg/kg)	Schedule	% TGI (Day 36)	TGD (days)
125	qwx3	79.8	22.5
150	biwx3	95.6	32.5
100	qd x5, 9d off x2	91.6	45

**Figure 5. SNS-314 shows strong anti-tumor activity in HCT116 xenografts on all dosing schedules tested.** Consistent and dose dependent activity observed across a panel of six additional xenograft models on a bi-weekly schedule.

## SNS-314 potentiates docetaxel's 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 inverse sequence (DTX → SNS-314, 24 hr separation) is not efficacious. Combination is not associated with additive toxicity compared to single agent treatments.