



THE PHASE I CLINICAL COMPOUND SNS-595 ACTS DURING S-PHASE AND CAUSES A SUSTAINED G2 ARREST

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ABSTRACT #2293

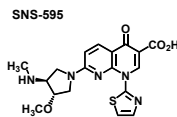
Although recent research has identified cancer therapies with non-cytotoxic mechanisms, there is still a need to discover and develop the next generation of cytotoxics with strong activities, manageable toxicities, and novel mechanisms of action. SNS-595 is a first-in-class naphthyridine compound that demonstrates potent cytotoxic activity as well as robust antineoplastic activity in several syngeneic and human xenograft tumor models, and is currently in phase I clinical trials for solid tumors. In vitro, SNS-595 has been observed to induce G2 cell cycle arrest and subsequent apoptosis in various cancer cells, including many multi-drug resistant lines. The effect of SNS-595 on cell cycle progression and checkpoint stimulation has been characterized and compared to the effects of a number of therapeutically relevant cell cycle modulators (cisplatin, docetaxel, gemcitabine, etoposide, doxorubicin, irinotecan, bleomycin, and mitomycin C). Cell cycle progression was analyzed using both DNA content and the cell cycle markers cyclins A, B, and E. In asynchronous populations, SNS-595 treatment caused a full G2 arrest in all cell lines tested. This arrest was accompanied by rapid apoptosis as determined by DNA fragmentation. In synchronized cell populations treated with SNS-595, cells cycled normally until they reach S phase, which was 30% longer than in untreated cells. Checkpoint markers (chk kinases, cdc25 phosphatases, cdc2, and p21) appeared rapidly upon entering S phase, and the cells eventually reached a sustained and irreversible arrest with 4N DNA content. SNS-595 is shown to be distinct from the other G2 arrestors tested in that it causes p21 expression early in S phase and a significant S phase lag. SNS-595 is also differentiable from other S-phase active compounds in showing a definitive arrest at G2 as opposed to a varied G1/S/G2 arrest profile. Further research into the stimulation of checkpoint and apoptotic pathways by SNS-595 will lead to a more detailed understanding of this compound's potent anticancer activity.

BACKGROUND

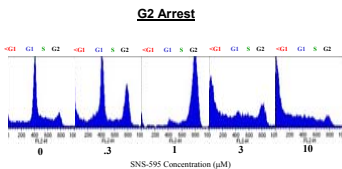
SNS-595, a naphthyridine derivative, is a novel cytotoxic agent intended for the treatment of several tumor types. SNS-595 has strong cytotoxic activity in vitro and in vivo in a wide range of human cancers. It is known to induce apoptosis in a cell cycle dependent manner using both p53 dependent and p53 independent pathways (abstract # 2285). The cytotoxic activity of SNS-595 has been demonstrated in more than 20 different tumor cell lines, and antitumor activity has been observed in 11 human xenograft tumor models and 3 syngeneic models in mice. SNS-595 has caused tumor regression, cell-cycle arrest, and apoptosis in these models (abstract #2277). Although the structure of SNS-595 resembles that of common quinolone antibiotics and cancer chemotherapeutics that target the topoisomerase family of enzymes, SNS-595 has a distinct activity profile from these drugs. Furthermore, cellular levels of topoisomerase II are not correlated with cytotoxicity of SNS-595, indicating that topoisomerase II is not the molecular target.

SNS-595 Cytotoxicity (subset of cell lines tested)

Cell Line	GI ₅₀ [μM]	MTT Viability Assay
HCT116 colon, carcinoma	256	
WDR colon, adenocarcinoma	884	
MES-SA uterus, sarcoma	276	
H1 brain, meningioma	748	
PC-14 non-small cell lung	2948	
HL-60 promyelocytic leukemia	186	
NCLH460 non-small cell lung	416	
PANCI-1 pancreas, carcinoma	309	
SKOV3 ovarian, carcinoma	1000	
SKOV3sp53 ^{-/-} ovarian, carcinoma	1200	



S-PHASE ACTIVITY: LEADS TO DISTINCTIVE G2 ARREST

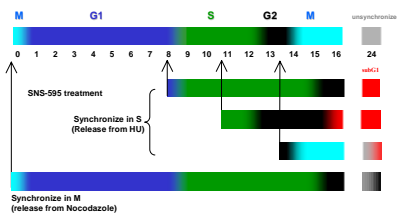


FACS analysis of HCT116 cells treated with SNS-595 for 16hrs at various concentrations. G2 arrest was determined by PI staining, and apoptosis by subG1 DNA content.

Cell Line	p53 Status	GI ₅₀ [μM]	MTT Viability Assay	EC ₅₀ [μM] G2 arrest
HCT-116	wt	256	620	120
HL-60	wt	186	120	900
NCLH460	wt	416	1600	1400
SKOV3sp53 ^{-/-}	wt	1200	1400	330
SKOV3	Null	1000	1400	
H1rat1	Mutant	54	36	
PANCI-1	Mutant	309	330	

Multiple cell lines of various p53 status display a dose dependent cytotoxicity, apoptosis and G2 arrest in response to SNS-595.

Cell Cycle Effects vs. Exposure Windows

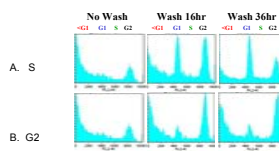


Graphical representation of FACS analysis. Shows synchronized HCT116 cells treated with 10μM SNS-595 during various cell cycle windows. Analysis was used to determine G2 arrest as well as subG1 cell populations as a function of time after SNS-595 exposure.

SNS-595 causes a G2 arrest if dosed before the G2 checkpoint, regardless of prior incubation time. A cell treated during G2/M will continue cycling and will arrest at the subsequent G2 phase. An S-phase lag is observed if SNS-595 is dosed before S-phase or early in S-phase.

G2 Arrest

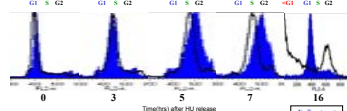
Irreversible Arrest



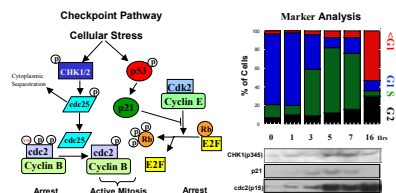
Synchronized HCT116 cells were treated with 10μM SNS-595 for either A. the majority of S-phase (9hrs), but removed before DNA-synthesis was complete or B. through S-phase until the G2 boundary was reached (9hrs). SNS-595 media was then aspirated and replaced by fresh media (5X over initial volume) and the cells were incubated for 1 hr (2 times). Fresh media was then added and the cells were allowed to cycle for 16hr and 36hrs after SNS-595 removal. Cell cycle recovery was measured by FACS analysis.

SNS-595 is an irreversible G2 arrestor if cells are treated with SNS-595 for the duration of DNA-synthesis and allowed to reach the G2 checkpoint before the drug is removed.

S-Phase Lag and Checkpoint Signaling



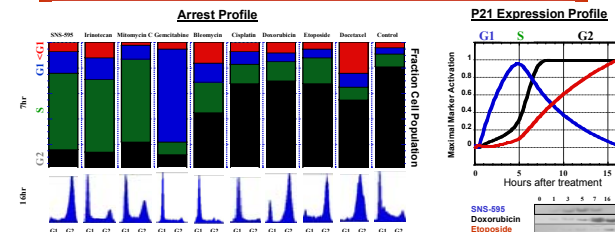
Synchronized HCT116 cells were treated with 10μM SNS-595 for various time points. Cell cycle progression was measured using FACS analysis and compared to untreated cells.



Synchronized HCT116 cells were treated with 10μM SNS-595 for various time points. Checkpoint markers were analyzed using western blot analysis and compared to cell cycle progression as determined by FACS analysis.

SNS-595 causes and S-phase lag in cycling cells. In the presence of SNS-595, checkpoint markers such as CHK1 phosphorylation, p21 expression and cdc2 phosphorylation(Y15) appear as cells enter S-phase.

NOVEL CELL CYCLE MODULATION PROFILE



Top: Synchronized HCT116 cells were treated with various cytotoxics for 7hrs after release from Hydroxyurea. Cells were then analyzed by FACS analysis for cell cycle progression. Bottom: Asynchronous HCT116 cells were treated with various cytotoxics for 16hrs, then analyzed by FACS analysis.

SNS-595 has a distinct response profile from studied therapeutics. Although it has an S-lag in synchronized cells similar to S-phase active compounds, it displays an exclusive G2 arrest when asynchronous cells are treated for one full cell cycle. Although SNS-595's G2 arrest profile mirrors that of typical G2 arrestors, the presence of an S-phase lag is novel. This S-phase lag is also accompanied by S-phase signaling, argin a hallmark of S-active compounds and not the known G2 arrest agents.

Comparator Table

	MOA	S lag	G2 Arrest	Signals in S-phase*
SNS-595	Unknown	+	+	+
Irinotecan	Topo I poison	+	-	+
Mitomycin C	Alkylator	+	-	+
Gemcitabine	Anti-metabolite	-	-	+
Bleomycin	DSB	-	-	+
Cisplatin	Cross-linker	-	-	+
Doxorubicin	TopoII poison/unknown	-	+	-
Etoposide	Topo II poison	-	+	-
Docetaxel	Tubulin inhibitor	-	-	-

* Signaling tested for all compounds: p21, cdc2-phosphorylation, CHK1 phosphorylation, as well as markers in p53 dependent and independent apoptosis (abstract #2295).

SUMMARY

- SNS-595
- S-Phase Active Agent
 - Displays and S-phase lag
 - Cell cycle checkpoints are activated at the onset of S-phase
- Irreversible G2 arrestor
- Novel Response Profile
 - S-phase lag and S-phase active pairs with other S-phase agents
 - Arrest profile most closely resembles typical G2 arrestors.

Conclusions:

In the cell models described here, SNS-595 acts exclusively during DNA synthesis; its cell cycle profile is clearly distinct from other cytotoxics, including topoisomerase inhibitors. These properties point to a novel mechanism of action for this phase I clinical compound.

We would like to thank Dr. George Stark for the generous use of his lab's SKOV3 matched cell lines +/- p53.

Cell Synchronization: Cells were synchronized at the G1/S boundary with 10mM Hydroxyurea treatment for 16hr. For synchronization in M Phase they were treated with 100ng/ml Nocodazole for 6hrs. After synchronization treatment, drug was removed and cells were rinsed with 6 volumes of fresh media over 2hrs. Drug treatments were then added and time courses were performed. For cell cycle window experiments drugs were dosed during specific phase windows as determine by FACS.

Western Blot: Cellular lysates were prepped and 10-20ug total protein(western dependent) was run on a 10% NuPage Bis-Tris Gel then transferred to a Nitrocellulose membrane and probed using 1st and 2nd mAb (p21-cellsignaling 2946, phos- p53(Y15) cellsignaling 9284, phos-p73(Y99) cellsignaling 4665, phos-cdc2(Y15)-Cellschem 219432).