



THE POTENT CYTOTOXIC AGENT SNS-595 CAUSES A RAPID ONSET OF APOPTOSIS DURING THE S-PHASE OF THE CELL CYCLE

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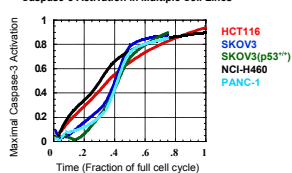
ABSTRACT #2285

SNS-595 TREATMENT LEADS TO RAPID APOPTOSIS

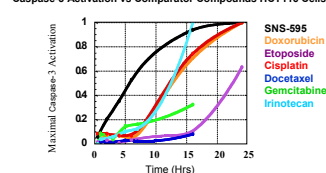
SNS-595: NOVEL S-PHASE CYTOTOXIC

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 cytotoxics (cisplatin, docetaxel, etoposide, gemcitabine, doxorubicin, irinotecan, bleomycin, and mitomycin C). The relationship between the cell cycle and apoptosis was studied in both asynchronous and synchronous 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 cytotoxics studied. Cells synchronized at G1/S 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 indicate that apoptosis is stimulated through p53-independent and dependent mechanisms. In contrast to comparator compounds, SNS-595 stimulates p21 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.

Caspase-3 Activation in Multiple Cell Lines

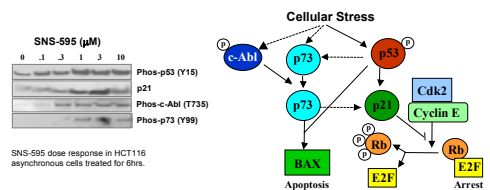


Caspase-3 Activation vs Comparator Compounds HCT116 Cells



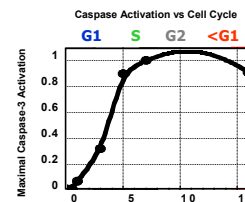
SNS-595 Treatment (10µM) leads to rapid Caspase-3 activation in multiple cell lines, independent of p53 status. In HCT116 cells, SNS-595 induces half maximal Caspase-3 activation within 5 hrs of treatment, 2-10X faster than other chemotherapies studied.

Dose Dependent Cellular Response



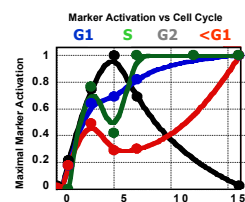
SNS-595 leads to an apoptotic response in a dose dependent manner. One can see p53 phosphorylation leading to p21 expression as SNS-595 concentrations are increased. SNS-595 also stimulates p53 independent apoptotic responses through c-Abl and p73 phosphorylation.

S-Phase Activity



Synchronized HCT116 cells dosed with SNS-595 (10µM) for various time points. Caspase-3 Activation measured by ELISA.

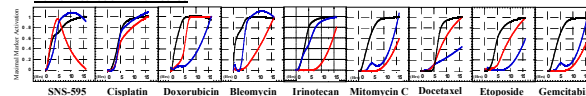
Caspase-3 Activation



Synchronized HCT116 cells dosed with SNS-595 (10µM) for various time points. Marker activation measured by western blot.

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 G2, M or G1 phases of the cell cycle, no matter what the incubation time (data not shown and abstract #2293).

Marker Activation Time Course

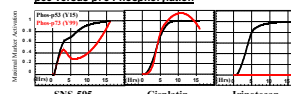


Phos-p53(Y15)

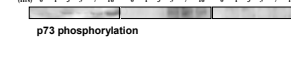
p21

Caspase-3 Activation

p53 versus p73 Phosphorylation



p73 phosphorylation



p73 phosphorylation

Synchronized HCT116 cells treated with various cytotoxics for the given time points. Lysates were then analyzed for marker activation using western blot analysis.

	MOA	p73 Phos	Cell Cycle Activity
SNS-595	Unknown	+	S
Cisplatin	Cross-linker	+	Independent
Doxorubicin	Topo II poison/unknown	+	Independent
Bleomycin	DSB inducer	+	Independent
Irinotecan	Topo I Poison	-	S
Mitomycin C	Abllyator	-	S
Etoposide	Topo II poison	-	G2
Docetaxel	Tubulin Inhibitor	-	M
Gemcitabine	Anti-metabolite	-	G1

SNS-595 has a distinct apoptotic profile:

- Though p53 dependent events occur, the biological activity of SNS-595 does not depend on p53
 - Caspase 3 activation in p53 null cells
 - phosphorylation of c-Abl and p73
- Rapid activation of later apoptotic, checkpoint and senescence markers
 - immediate p21 expression
 - rapid caspase-3 activation
- p73 phosphorylation response – not common to all cytotoxics

SUMMARY

SNS-595:

- 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.

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

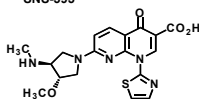
BACKGROUND

SNS-595, a naphthyridine derivative, is a novel cytotoxic agent intended for the treatment of several tumor types. It is a cell cycle modulator that causes an S-phase lag leading to an irreversible G2 arrest and rapid apoptosis (abstract #2283). SNS-595 has strong cytotoxic activity in vitro and in vivo in a wide range of human cancers. 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 tumors, and 3 syngeneic cancer models. SNS-595 has caused tumor regression, cell-cycle arrest, and apoptosis in these models (abstract #2277).

SNS-595 Cytotoxicity (subset of cell lines tested)

	GI ₅₀ [nM] MTT Viability Assay
HCT116 colon, carcinoma	256
WiDr colon, adenocarcinoma	804
MES-SA uterus, sarcoma	276
H4 brain, neuroglioma	940
PC-14 non-small cell lung	2940
HL-60 promyelocytic leukemia	106
NCI-H460 non-small cell lung	416
PANC-1 pancreas, carcinoma	309
SKOV3 ovarian, carcinoma	1000
SKOV3(p53 ^{-/-}) ovarian, carcinoma	1200

SNS-595



METHODS

Cell Synchronization: Cells were synchronized at the G1/S boundary with 10nM Hydroxyurea treatment for 16hr. For synchronization in M phase they were treated with 100ng/ml Nocodazole for 8hrs. After synchronization treatment, drug was removed and cells were rinsed with 6 volumes of fresh media over 2hrs.

Caspase 3 ELISA: Drug treatments were then added and time courses were performed. Cellular lysates were prepared and 50µg total protein was used in a capture ELISA. Maxisorp Plate -anti-caspase-3 (BD 610322) -> lysate -> anti-cleaved caspase-3 (cellsignaling 9661) -> 2nd mAb-HRP (Chemicon)

Western Blot: Cellular lysates were prepared and 10-20µg total protein (western dependent) was run on 10% NuPage Bis-Tris Gel and then transferred to a Nitrocellulose membrane and probed using 1: and 2: mAb (p21 cellsignaling 2946, phos-p53(Y15) cellsignaling 9284, phos-p73(Y99) cellsignaling 4665, phos-c-Abl(T735) cellsignaling 2864).