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

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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 antiangioplastic 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 multidrug-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 (caspatin, docetaxol, gemcitabine, etoposide, doxorubicin, bleomycin, and mitomycin C). Cell cycle progression was analyzed using both DNA content and the cell cycle markers cyclin 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 (cdk kinases, cdk25 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 striking cytotoxic activity in vitro and in vivo in a wide range of human cancer cells. It is known to induce apoptosis in a cell cycle dependent manner using both p53 dependent and p53 independent pathways [1,2,3]. The cytotoxicity 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 vivo. SNS-595 has caused tumor regression, cell cycle arrest, and apoptosis in a number of xenograft and syngeneic models. SNS-595 is a potent inducer of apoptosis in Jurkat cells that overexpress Bcl-2, and is unique among 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.

METHODS

SNS-595 Cytotoxicity (subset of cell lines tested)

SNS-595 causes a G2 arrest if dosed before the G2 checkpoint, regardless of prior incubation time. A cell treated during G2M 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.

NOVEL CELL CYCLE MODULATION PROFILE

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, again a hallmark of S-active compounds and not the known G2 arrest agents.

SUMMARY

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.