**Abstract**

Aurora kinase inhibitors constitute a family of serine-threonine kinases that are strongly associated with cancer. Aurora A and B are essential in mitosis. Perturbation of their activity leads to multiple defects in mitosis including aberrant centrosome duplication, misalignment of chromosomes, inhibition of cytokinesis, and disruption of the spindle checkpoint. The role of Aurora C is unclear, however. Aurora C can complement Aurora B kinase activity in mice. SNS-314 is an ATP-competitive, selective, and potent nanomolar inhibitor of Aurora kinases in vitro. A co-crystal structure of SNS-314 with Aurora A confirms that SNS-314 engages the punit-binding pocket of Aurora. SNS-314 inhibits cellular proliferation in the HCT116 colorectal carcinoma cell line with an IC_{50} of ~5 nM. Analysis of DNA content and indirect immunofluorescence demonstrates that SNS-314 induces defects in cytokinesis and spindle checkpoint that are consistent with Aurora kinase inhibition. Phosphorylation of Histone H3 on serine 10, a known Aurora B cellular target, is inhibited with an EC_{50} = 9 nM. Analysis of DNA content and indirect immunofluorescence demonstrates that SNS-314 treated tumors confirm that the anti-tumor activity is consistent with Aurora kinase inhibition. SNS-314 is a novel, small-molecule inhibitor of Aurora kinase that is being developed as a novel anti-cancer therapeutic agent.

**Background**

SNS-314 is a novel, small-molecule (~ 450 Da) multi-Aurora kinase inhibitor. Aurora kinase activity is central to mitosis and cytokinesis, and shutdown of their activity leads to aberrant mitotic cell division, aneuploidy, and apoptosis. Thus, a small-molecule antagonist of Aurora kinase activity is predicted to be an effective anti-cancer agent. SNS-314 exhibits potent anti-tumor activity in multiple xenograft models and will enter phase 1 clinical trials for the treatment of patients with advanced solid tumors in 2007.

**Methods**

**Kinase assays:** SNS-314 was tested for inhibitory activity against a panel of 219 kinases (Echelon Biotechnology, Dundee, UK). All screens were performed by incubating the kinase, SNS-314, and radiolabeled ATP for typically 30-60 minutes. The final ATP concentration in the reaction was within 15 nM of the K_{i} for ATP, as calculated by Upstate. Kinase biochemical assays: A Homogenous Time-Resolved Fluorescence (HTRF)-based biochemical IC_{50} assay from Cisbio (Bedford, MA) was used to test for the kinase activity of the kinase isoforms of Aurora–Aurora A, B, and C– in the presence of SNS-314. A biotin conjugated histone H3 phosphate (Upstate Biotechnology) was used as a substrate.

**Crystallography:** Diffraction-qualifying crystals of Aurora-A in complex with inhibitors were obtained by hanging-drop vapor diffusion at 20-25°C. Diffraction data were collected under standard cryogenic conditions on X-rays. All crystals were processed according to standard procedures. Images were captured and pHH3 was analyzed using the Target Activation application and ArrayScan VTI instrument (Cellomics, Inc.). Data points taken from the parameter Mean_AveIntenCh2 were graphed in GraphPrism and fitted into an IC_{50} equation. Fluorescent imaging: Slides were examined on a LEICA DMIRE2 fluorescent microscope with a 63x oil immersion objective. Images were captured using a LEICA DFC300FX CCD camera and analyzed using Image-Pro software.

**Pharmacology:** Mice (nu/nu) were subcutaneously implanted with HCT-116 colorectal carcinoma cells in the right hind flank with 200 μl of a 2.5 × 10^6 tumor cell suspension (1:1 PBS with cells/ Matrigel). When tumors reached an average volume of 198 mm³, mice were randomized into groups and treated with vehicle or SNS-314. All animal experiments were in accordance with protocols approved by the Sanusis Pharmaceuticals, Inc. Institutional Animal Care and Use Committee and in accordance with local state and federal regulations. Following staining with hematoxylin and eosin, sections were deparaffinized, rehydrated, sectioned, and transferred to slides. Tumor sections were stained with hematoxylin and eosin (H&E).

**Summary of IC_{50} data for SNS-314 versus Aurora A, B, and C.**

**SNS-314 blocks proliferation in a broad panel of tumor cell lines**

**SNS-314 shows potent anti-tumor activity consistent with Aurora kinase inhibition**

**Summary and Conclusions**

SNS-314 is a highly selective and potent multi-Aurora kinase inhibitor that binds to the active form of Aurora in an extended conformation. SNS-314 shows low nanomolar anti-proliferative activity in a broad panel of cancer cell lines. SNS-314 demonstrates potent anti-tumor activity in a human colorectal carcinoma model that is consistent with Aurora B inhibition as the mechanism of action. The combination of potency, selectivity, and robust in vivo activity coupled to flexible intermittent dose schedule suggest that SNS-314 may be a best in class Aurora kinase inhibitor for the treatment of diverse human malignancies.

An IND has been submitted for SNS-314 and a phase 1 clinical trial for the treatment of patients with advanced solid tumors is planned in 2007.