SNS-062 is a potent noncovalent BTK inhibitor with comparable activity against wild type and mutant BTK

SNS-062 demonstrated a clear PK/PD relationship in DBA/1 mice as evidenced by increasing inhibition of pBTK with increasing SNS-062 exposure. With an observed IC50 for pBTK inhibition at a plasma concentration of 47 nM (equivalent to 25 ng/ml), and complete inhibition of pBTK observed at ~100 nM (53 ng/ml).

- BTK phosphorylation levels in cells expressing BTK C481S was minimally affected by the C481S mutation
- BTK activity of SNS-062 was not affected by mutation of C481 in the kinase active site
- SNS-062 demonstrated clear normal oral bioavailability in rat, dog and monkey (18%, 65%, 33%). Clearance was low to moderate and Vd was low, 112 ml/kg 3–6 hours
- SNS-062 had no adverse effects on cardiovascular or nervous systems in safety pharmacology studies. In 28-day definitive toxicology studies in rat and dog, oral once-daily administration of SNS-062 was well tolerated with continuous exposure
- PK, PD and toxicity studies demonstrated that plasma concentrations anticipated to provide VD inhibition of BTK could be sustained with acceptable tolerability

Methods

- Crystallography: recrystallized human BTK kinase domain (BTK KD, AA 402-699) expressed and purified as described, was mixed with SNS-062 and evaluated by crystallization using sitting drop vapour diffusion. BTK KD, SNS-062 complex crystal and data to fullest resolution were processed with MOSFLM and numeric software. 
- FPLC purification: Purification and characterization were performed on Q-Sepharose and Superdex 200 using a 10mL FPLC system (GE Healthcare, USA) and a 20mL FPLC system (GE Healthcare, USA), respectively.
- Activity assay: The activity assay was performed in the presence of 50 μM ATP and p-benzoyl-tyrosine amide (BAPA) as described.
- Western blotting: SDS-PAGE gel and Western blot analysis were performed as described.

References