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SM08502, a novel, small-molecule CDC-like kinase (CLK) inhibitor, demonstrates activity against cancer stem cell (CSC)-enriched pancreatic cancer cells and suppresses stemness *in vitro*

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Background: Cancer stem cells (CSCs) are a rare subpopulation of quiescent tumor cells with the ability to self-renew and form new tumors (stemness) that have been identified in many tumor types, including pancreatic cancer (PC). The presence of CSCs in PC tumors may contribute to chemotherapy resistance and relapse. The Wnt signaling pathway is a highly conserved developmental pathway that has been implicated in the maintenance and survival of CSCs. Aberrant activation of Wnt signaling is common in PC where it may functionally support cell proliferation and tumor forming capacity; therefore, this pathway is a therapeutic target of interest. SM08502 is a novel, small-molecule pan-CLK inhibitor that has been shown to potently inhibit Wnt pathway activity in preclinical studies. The purpose of these studies was to test the hypothesis that SM08502 could impair CSC viability and stemness in PC cell lines.

Methods: CSC-enriched tumor spheres were generated by culturing Panc1 cells in nonadherent conditions in which most cells lacking stemness undergo anoikis (programmed cell death). These CSC-enriched Panc1 (Panc1-CSC) cell cultures were analyzed for stemness marker expression via FACS and qPCR to validate the enrichment protocol. The effect of SM08502 on spheroid formation and viability was assessed in comparison to salinomycin and napabucasin as positive controls for CSC inhibition. Inhibition of CSCs within parental PC lines (Capan-1, HPAFII, and Panc1) was assessed by 4-day pretreatment of cells in culture with DMSO or SM08502 and a 4-day recovery period followed by plating 1 or 10 cells/well in 96-well plates under nonadherent conditions and quantifying spheroid formation after 24 days.

Results: Anoikis-mediated CSC enrichment increased the percentage of cells expressing the stem cell surface protein marker CXCR4 and increased expression of multiple stemness-related genes. Treatment with either positive control compounds or SM08502 inhibited spheroid formation and decreased the viability of the Panc1-CSC cells. Notably, SM08502 was more potent by EC_{50} than either salinomycin or napabucasin in the cell viability assay. Expression analyses indicated that SM08502 significantly reduced stemness-related gene expression in Panc1-CSC cultures, including *CD24*, *LGR4*, and *VDR* ($p<0.01$ vs DMSO). In addition, treatment of Capan-1, HPAFII, and Panc1 cells with SM08502 strongly impaired their ability to form spheres by 8- to 12-fold vs. DMSO, indicating that CSCs within these cell line populations are sensitive to treatment with SM08502.

Conclusion: In summary, SM08502 demonstrated anti-CSC activity against PC and appeared more potent than known CSC-inhibiting compounds. By depleting CSCs and reducing stemness in tumors, SM08502 can potentially address relapse and treatment resistance in PC. A Phase 1 study assessing safety, tolerability, and pharmacokinetics of SM08502 in subjects with advanced solid tumors is ongoing (NCT03355066).