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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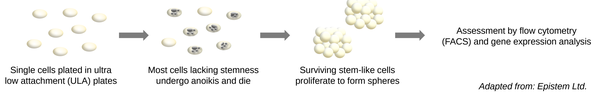
Poster #C08

Background

- Cancer stem cells (CSCs) are a rare subpopulation of quiescent tumor cells with stemness, the ability to self-renew and form new tumors, and may contribute to chemotherapy resistance, proliferation, and relapse in pancreatic cancer (PC)[1,2]
- Aberrant activation of the Wnt signaling pathway is implicated in multiple cancer hallmarks including proliferation, metastasis, and immune evasion, as well as the maintenance and survival of CSCs[1,3]
- CDC-like kinases (CLKs) phosphorylate serine/arginine-rich splicing factors (SRSFs), which regulate spliceosome assembly and subsequent gene expression[4,5]
- SM08502 is a novel, oral, small-molecule pan-CLK inhibitor that has been shown to potently inhibit Wnt pathway activity in preclinical colorectal cancer models[6]
- These studies examined the ability of SM08502 to impair CSC viability and stemness in PC cell lines

Methods

• Panc-1 cell cultures were enriched in CSCs (Panc1-CSC) by inducing anoikis, programmed cell death triggered by non-adherent growth conditions (**Fig. 1**):



Adapted from: Epistem Ltd.

•Cell (spheroid) viability – Panc1-CSC cultures were plated on day zero (D0) in ULA plates and incubated until D3. On D3, the nascent spheroids were treated with salinomycin, napabucasin (both CSC-inhibiting positive controls[7,8]), or SM08502 until D6, and viability was assessed via CellTiter-Glo 3D™ luminescence assay (**Fig. 2**)

•Spheroid formation – Panc1-CSC cultures were plated on day zero (D0) in ULA plates and treated on D1 with salinomycin, napabucasin, or SM08502 for 6 days, after which the plates were imaged using a CellInsight™ CX5 imager (**Fig. 3**)

• Gene expression – qRT-PCR after 20 h exposure to vehicle or SM08502 (1 µM) using TaqMan® primers and normalizing expression to GAPDH (via $\Delta\Delta$Ct); stemness-associated genes were assessed in both Panc-1 and Panc1-CSC cultures (**Fig. 4**)

• Spheroid-forming frequency – HPAFII, Capan-1, and Panc-1 parent cell cultures were plated on D0 in 6-well plates and treated with SM08502 (1 µM) or vehicle per the timeline below. Spheroid-containing wells were counted around D24 (**Fig. 5**)

Results

Figure 1. Panc-1-enriched cells demonstrated increased expression of the stem cell surface marker CXCR4

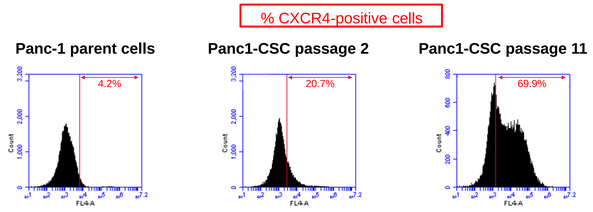
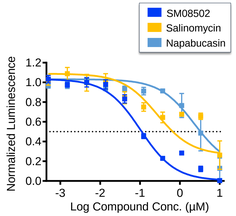


Figure 2. SM08502 potently impaired Panc1-CSC spheroid viability



	SM08502	Salinomycin	Napabucasin
EC_{50} (µM)	0.1028	0.2682	2.301
Span	1.031	0.8255	0.9032

Figure 3. SM08502 impaired formation of Panc1-CSC spheroids

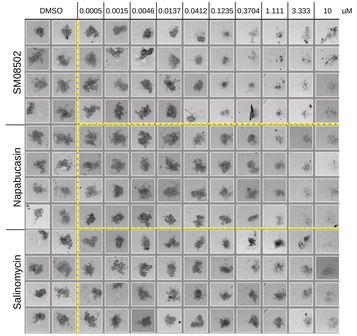
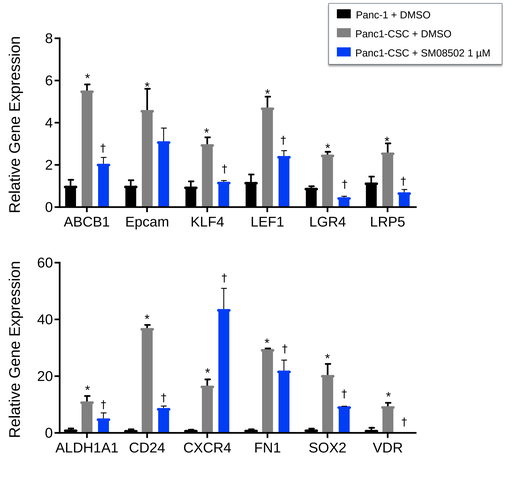
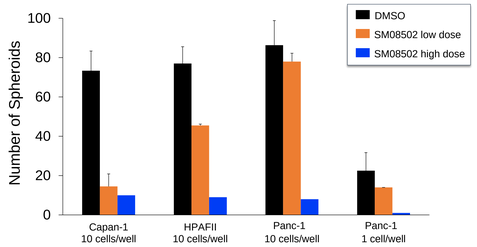


Figure 4. SM08502 reduced stemness-related gene expression in Panc1-CSC spheroids



*p<0.05 vs. Panc-1 DMSO †p<0.05 vs. Panc1-CSC DMSO

Figure 5. SM08502 dose-dependently inhibited frequency of spheroid formation in PC parental cell lines



SM08502	Capan-1	HPAFII	Panc-1
Low dose (µM) (n=2)	0.3	0.3	0.35
High dose (µM) (n=1)	0.4	0.4	0.39

Conclusions

- **CSCs were successfully enriched in Panc-1 parent cell cultures**
- **SM08502 demonstrated strong activity against CSCs in pancreatic cancer cell lines**
- **SM08502 anti-CSC activity was more potent than other CSC inhibitors (salinomycin and napabucasin) *in vitro***
- **SM08502 inhibited the stemness of CSCs and parental PC cells**
- **SM08502 can potentially address relapse and treatment resistance in PC by depleting CSCs and reducing stemness in tumors**
- **A Phase 1 study assessing the safety, tolerability, and pharmacokinetics of SM08502 in subjects with advanced solid tumors is ongoing (NCT03355066)**

References

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Other disclosures are listed in the published abstract.

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