SM08502, a novel, small-molecule CDC-like kinase (CLK) inhibitor, downregulates the Wnt signaling pathway and demonstrates antitumor activity in pancreatic cancer cell lines and *in vivo* xenograft models

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Poster #A02

Background

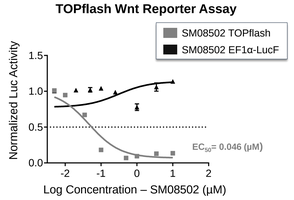
- Aberrant activation of the Wnt signaling pathway is implicated in multiple cancer hallmarks including proliferation, metastasis, and immune evasion[1]
- This may also promote fibrogenesis in the tumor microenvironment characterized by stroma, which contributes to treatment resistance[2,3]
- CDC-like 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 demonstrated potent Wnt signaling inhibition in preclinical colorectal cancer models[6]
- These studies examined *in vitro* and *in vivo* activity of SM08502 in preclinical models of pancreatic cancer (PC)

Methods

- *In vitro* assays:
  - Wnt pathway inhibition – Expression of the Wnt-responsive TOPflash or EF1α-luciferase reporter gene constructs in cells (plated at ~10^4 cells/well) incubated for 40 h in DMSO (vehicle) or SM08502 (**Fig. 1**)
  - CLK inhibition – Z'-LYTE™ kinase assays (**Fig. 1**)
  - Cell viability – CellTiter-Blue® fluorescence assay (**Table 1**)
  - 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) (**Fig. 2**)
  - Colony formation – Cells were plated (1-5 x 10^3 cells/well) and incubated with vehicle or SM08502 for 7 days; the colonies were then labeled with crystal violet (**Fig. 3**)
  - Apoptosis – Caspase-Glo® 3/7 detection assays on cultures were incubated overnight and then treated with vehicle, SM08502, or staurosporine at 37°C for 48 h (**Fig. 4**)
  - All assays except the Wnt pathway and CLK inhibition assays utilized Capan-1, HPAFII, and Panc-1 PC cell lines
- *In vivo* assays:
  - Mouse xenografts – Nude mice were implanted in the right flank with Capan-1 or HPAFII cells and randomized into treatment groups when tumors reached ~100-200 mm³. Mice were orally treated with vehicle or SM08502 for 21 days and tumor growth inhibition (TGI) was calculated relative to vehicle
  - Regressions were assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines: 30-100% reduction in tumor volume relative to the start of the study. Tolerability was determined by average bodyweight change from baseline (<15% loss was considered well tolerated) (**Figs. 5-6**)
  - Stroma modeling – PC cells were implanted as above with or without cancer-associated fibroblasts (CAFs; VitroBiopharma CAF08 cell line or Bio-IVT primary human fibroblasts). TGI was calculated relative to respective vehicle group (**Fig. 6**)

Results

Figure 1. SM08502 is a potent CLK inhibitor that inhibits Wnt signaling *in vitro*



CLK Inhibition Assay

	CLK2	CLK3	CLK1	CLK4	CDK1
IC_{50} (µM)	0.002	0.022	0.008	0.001	1.06

Table 1. SM08502 inhibited pancreatic cancer cell proliferation *in vitro*

KRAS Status	Cell Line	Mutation Profile	EC_{50} (µM)	Average EC_{50} (µM)
Mutation	**Capan-2**	KRAS	0.526	
	HPAFII	KRAS,TP53,CDKN2A	0.098	
	MIA Paca-2	KRAS,TP53,CDKN2A	0.078	
	PANC-1	KRAS,TP53,CDKN2A	0.126	
	Su.86.86	KRAS,TP53, CDKN2A	0.188	
	YAPC	KRAS,TP53, SMAD4	0.155	**0.222**
	HPAC	KRAS, CDKN2A, SMAD4	0.223	
	Capan-1	KRAS, TP53, CDKN2A, SMAD4	0.253	
	AsPC1	KRAS, TP53, CDKN2A, SMAD4	0.155	
	CFPAC1	KRAS, TP53, CDKN2A, SMAD4	0.490	
	Hs766T	KRAS, TP53, CDKN2A, SMAD4	0.155	
Wild Type	**BxPC3**	TP53, CDKN2A, SMAD4	0.211	
	Hs700T	TP53	0.222	**0.168**
	SNU-324	CDKN2A	0.072	
		All Cell Lines		**0.211**

Figure 2. SM08502 inhibited Wnt pathway-related gene expression *in vitro*

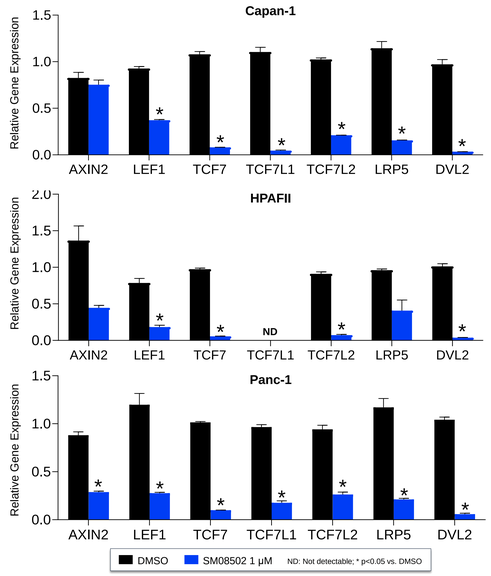


Figure 3. SM08502 inhibited cell colony formation

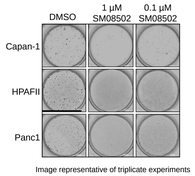


Image representative of triplicate experiments

Figure 4. SM08502 induced apoptosis

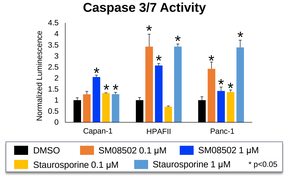
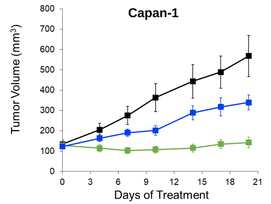
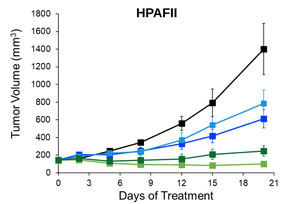


Figure 5. SM08502 inhibited tumor growth in PC xenografts





Capan-1	TGI	p-value	Regressions
Vehicle, QD	0%	1.000	0/6
SM08502 12.5 mg/kg QD	40%	0.075	0/6
SM08502 25 mg/kg QD	**75%**	**0.007**	**1/6**

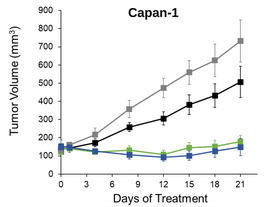
n=6 mice/group; T-test, two-tailed, assume unequal variance; p-value vs. vehicle
Bold indicates significance

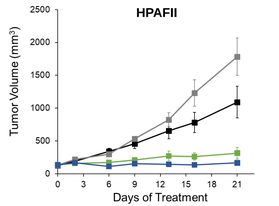
HPAFII	TGI	p-value	Regressions
Vehicle, QD	0%	1.000	0/5
SM08502 12.5 mg/kg QD	**56%**	**0.051**	0/5
SM08502 25 mg/kg QD	**93%**	**0.011**	**3/5**
SM08502 12.5 mg/kg QOD	44%	0.109	0/5
SM08502 25 mg/kg QOD	**82%**	**0.015**	0/5

n=5 mice/group; T-test, two-tailed, assume unequal variance; p-value vs. vehicle
Bold indicates significance

Average bodyweight loss was <5% from baseline with SM08502 treatment for Capan-1 and HPAFII xenografts

Figure 6. SM08502 induced tumor growth inhibition in PC xenografts with and without CAF co-implantation





Capan-1	Treatment	TGI	p-value	Regressions
Capan-1	Vehicle	0%	1	0/9
Capan-1	SM08502 25 mg/kg	**65%†**	**0.005**	**1/9**
Capan-1 + CAFs	Vehicle	-44%†	0.421	0/5
Capan-1 + CAFs	SM08502 25 mg/kg	**80%‡**	**0.005**	**1/5**

n=9 or 5 (+CAF) mice/group; T-test, two-tailed, assume unequal variance
Bold indicates significance; † vs. vehicle, ‡ vs. vehicle + CAFs

HPAFII	Treatment	TGI	p-value	Regressions
HPAFII	Vehicle	0%	1	0/6
HPAFII	SM08502 25 mg/kg	**71%†**	**0.022**	**1/6**
HPAFII + CAFs	Vehicle	-63%†	0.09	0/6
HPAFII + CAFs	SM08502 25 mg/kg	**85%‡**	**0.002**	**1/6**

n=6 mice/group; T-test, two-tailed, assume unequal variance
Bold indicates significance; † vs. vehicle, ‡ vs. vehicle + CAFs

Average bodyweight loss was <5% from baseline with SM08502 treatment for Capan-1 and HPAFII xenografts

Conclusions

- **SM08502 exhibited *in vitro* antitumor activity in multiple PC cell lines**
- **SM08502 was active regardless of status of *KRAS*, a mutation that is present in >90% of PC tumors**
- **SM08502 induced apoptosis**
- **SM08502 exhibited potent *in vivo* antitumor activity**
- **Tumor growth was strongly inhibited with daily and intermittent dosing regimens**
- **The presence of CAFs in xenografts did not affect the activity of SM08502, suggesting that SM08502 could potentially overcome the tumor-protective effects of stroma in PC tumors**
- **SM08502 was well tolerated**
- **Together, these data suggest that SM08502 has potential as a novel treatment of pancreatic cancer**
- **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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