SM08502, a novel, small-molecule CDC-like kinase (CLK) inhibitor, demonstrates strong antitumor effects and Wnt and cyclin D-CDK4/6-RB pathway inhibition in hormone-receptor-positive (HR+) breast cancer models

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Poster #6401

Background

- Dysregulation of the cyclin D-CDK4/6-RB signaling axis is implicated in HR+ breast cancer (BC)[1]
- While CDK4/6 inhibitors such as palbociclib (Palbo) have shown efficacy in this cancer type, overcoming resistance to these agents is an unmet need for patients
- CLKs regulate activity of serine/arginine-rich splicing factors (SRSFs), which modulate spliceosome assembly, mRNA splicing, and gene expression[2,3]
- SM08502, a pan-CLK inhibitor, has demonstrated strong antitumor activity in several preclinical cancer models and has been shown to inhibit the Wnt pathway via disruption of alternative splicing[4–7]
- We examined SM08502 activity in preclinical models of CDK4/6 inhibitor-sensitive and -resistant HR+, HER2-negative (HER2-) BC

Conclusions

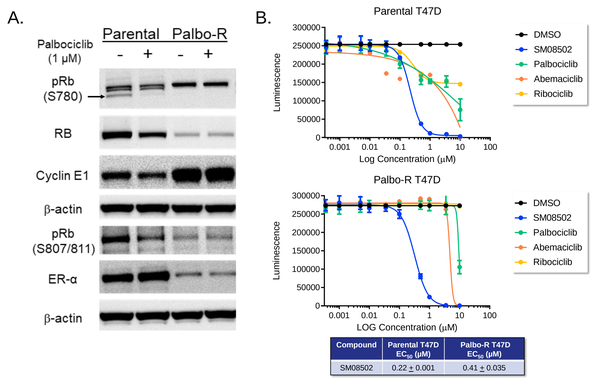
- **SM08502 strongly inhibited SRSF phosphorylation, Wnt-related gene expression, cell proliferation, and RB pathway signaling in multiple HR+, HER2- breast cancer cell lines**
- ***In vivo*, SM08502 demonstrated strong antitumor effects in MCF7 xenografts and HR+ PDX models**
- **Together, these data suggest that SM08502 has potential antitumor activity in HR+ BC and may provide clinical benefit to patients as a single agent or combined with standard therapy**
- **A Phase 1 study of SM08502 in subjects with advanced solid tumors is ongoing (NCT03355066)**

Results

Table 1. SM08502 impaired cellular proliferation of BC cell lines regardless of subtype

Subtype	Cell Line	EC_{50} (μM)	Average EC_{50} (μM)
Luminal A (HR+, HER2-)	MCF7	0.128	0.138
	T47D	0.147	
Luminal B (HR+, HER2+)	ZR-75-1	0.510	0.294
	BT474	0.261	
	MDA-MB-361	0.110	
HER2 (HR-, HER2+)	SK-BR-3	0.058	0.059
	MDA-MB-453	0.037	
	JIMT-1	0.082	
TNBC	MDA-MB-157	0.191	0.142
	MDA-MB-231	0.143	
	MDA-MB-468	0.117	
	BT-549	0.240	
	BT-20	0.167	
	CAL-51	0.055	
	Hs 578T	0.080	
Average EC_{50} of All Cancer Cell Lines		**0.155**	
Normal Breast Cells	Hs578Bst	1.517	

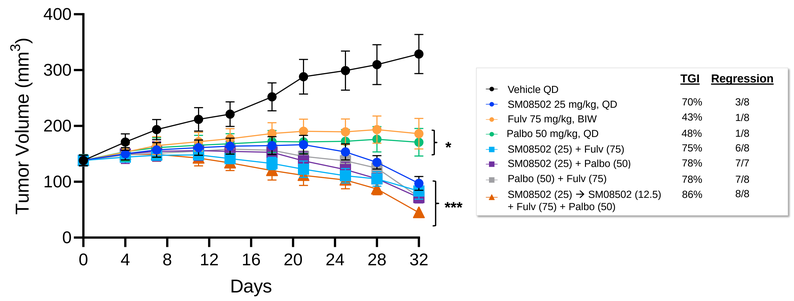
Figure 2. SM08502 inhibited proliferation and the RB pathway in Palbo-R T47D cells compared with CDK4/6 inhibitors



Compound	Parental T47D EC_{50} (μM)	Palbo-R T47D EC_{50} (μM)
SM08502	0.22 ± 0.001	0.41 ± 0.035

A. 6-hour treatment **B.** 6-day viability assay; n=2–4 independent studies; Mean ± SEM **C.** 48-hour treatment

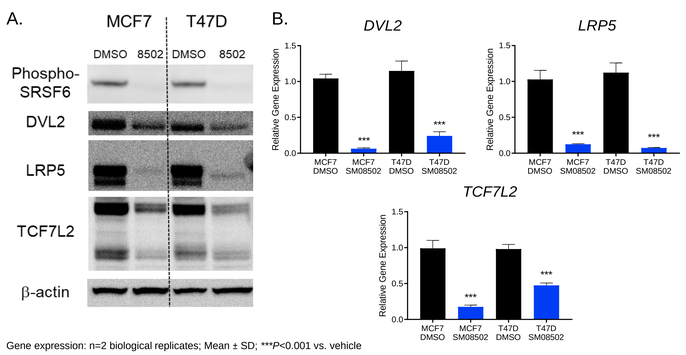
Figure 4. SM08502 ± SOCs induced tumor regression in MCF7 xenografts



	TGI	Regression
Vehicle QD		
SM08502 25 mg/kg, QD	70%	3/8
Fulv 75 mg/kg, BIW	43%	1/8
Palbo 50 mg/kg, QD	48%	1/8
SM08502 (25) + Fulv (75)	75%	6/8
SM08502 (25) + Palbo (50)	78%	7/7
Palbo (50) + Fulv (75)	78%	7/8
SM08502 (25) → SM08502 (12.5) + Fulv (75) + Palbo (50)	86%	8/8

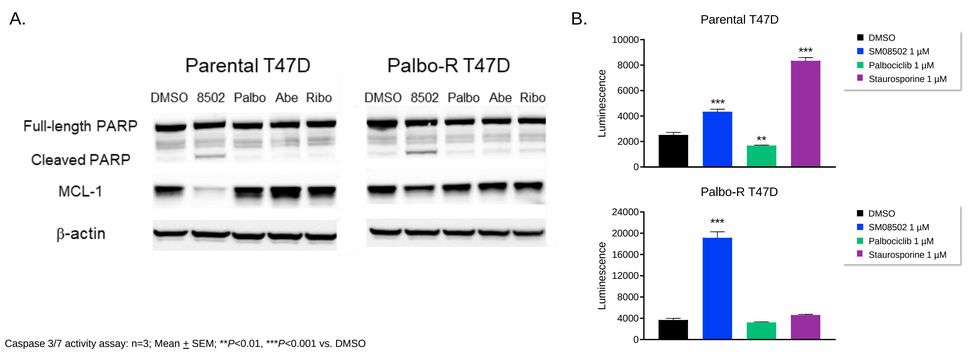
MCF7 tumor growth; n=7–8 per group; Mean tumor volume ± SEM; *P<0.05, ***P<0.001 vs. vehicle by repeated-measures analysis of variance

Figure 1. SM08502 inhibited SRSF6 phosphorylation and Wnt pathway-related protein and gene expression in HR+, HER2- cell lines



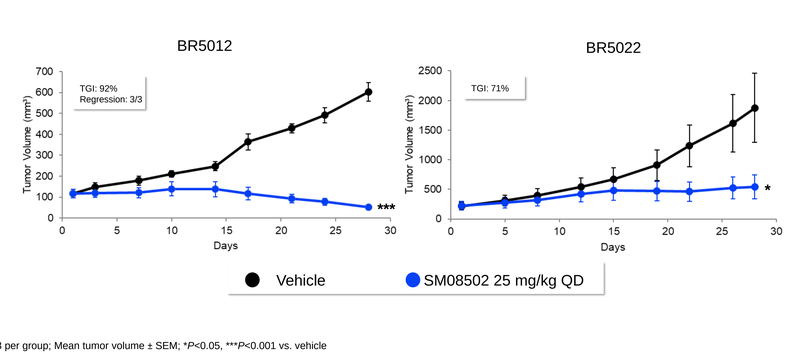
Gene expression: n=2 biological replicates; Mean ± SD; ***P<0.001 vs. vehicle

Figure 3. SM08502 induced apoptosis in Palbo-R cells compared with CDK4/6 inhibitors



Caspase 3/7 activity assay: n=3; Mean ± SEM; **P<0.01, ***P<0.001 vs. DMSO

Figure 5. SM08502 demonstrated strong antitumor activity in HR+ PDX models



n=3 per group; Mean tumor volume ± SEM; *P<0.05, ***P<0.001 vs. vehicle

Methods

In vitro assays

- Cellular proliferation was assessed in 15 BC cell lines following 4 days of treatment using the CellTiterBlue® assay (**Table 1**)
- Effects of SM08502 (1 μM) on SRSF phosphorylation and Wnt pathway-related protein and gene expression after 24 hours of treatment were measured by Western blot (**Fig. 1A**) and qRT-PCR (**Fig 1B**), respectively. Relative gene expression was determined by normalizing to GAPDH
- Palbo-resistant (Palbo-R) T47D cells were characterized by Western blot (**Fig 2A**). Effects of 1 μM SM08502, palbociclib, abemaciclib, or ribociclib on cell proliferation and the RB pathway in parental and Palbo-R T47D cells was assessed by CellTiterGlo ® assay (**Fig. 2B**) and Western blot (**Fig. 2C**)
- Apoptosis in cells treated with 1 μM SM08502, Palbo, abemaciclib (Abe), ribociclib (Ribo), or staurosporine for 48 hours was assessed by Western blot (PARP cleavage and expression of MCL-1) (**Fig. 3A**) and the Caspase-Glo® 3/7 assay kit (**Fig. 3B**)

In vivo assays

- Cell line-derived xenografts: Severe combined immunodeficient (SCID) mice were implanted with MCF7 cells in the right flank and randomized into treatment groups when tumors reached ~100–200 mm^3. Mice were orally treated with SM08502 or Palbo or subcutaneously injected with fulvestrant (Fulv) for indicated times, combinations, and doses (**Fig. 4**)
- Patient-derived xenograft (PDX): SCID mice were subcutaneously implanted with a patient-derived tumor fragment (BR5012 and BR5022, CrownBio) in both flanks (**Fig. 5**)
  - Tumor growth inhibition (TGI) was calculated relative to vehicle
  - Tumor regressions were assessed according to the 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 considered tolerated)

References

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All authors are employees, shareholders, or consultants of Samumed, LLC. Other disclosures are listed in the published abstract.

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