BWC5167 explores spliceosome-driven therapeutic potential in pancreatic cancer

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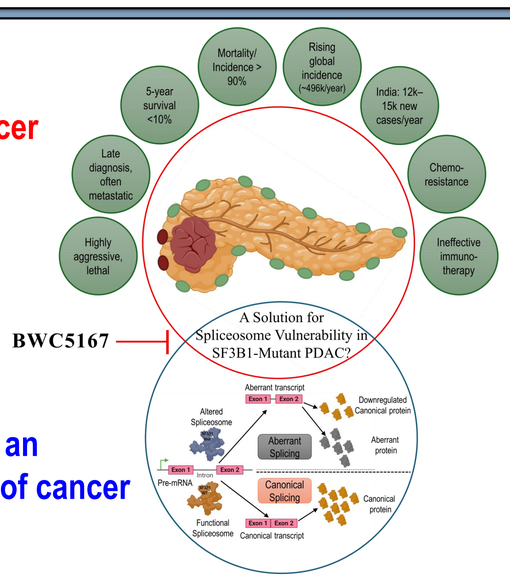
Abstract

Aberrant RNA splicing is a recognized hallmark of cancer, yet its therapeutic potential in solid tumours like **pancreatic ductal adenocarcinoma (PDAC)** remains poorly characterised in clinical and translational settings. We evaluated **BWC5167**, a novel spliceosome modulator, in five PDAC cell lines with distinct **SF3B1 (splicing factor 3B subunit 1) backgrounds**. BWC5167 **selectively induced apoptosis** and reduced viability in **SF3B1-mutant lines** (Panc 05.04 and Capan-1), with negligible effects in wild-type cells. Mechanistically, BWC5167 increased **intron retention**, downregulated **phospho-SF3B1**, **SRSF1**, and **c-Myc**, confirming spliceosome targeting. These results highlight **spliceosome vulnerability in SF3B1-mutant PDAC**, positioning BWC5167 as a promising precision oncology candidate warranting further transcriptomic and in vivo validation.

Introduction

PDAC- A lethal cancer on the rise



Aberrant splicing – an emerging hallmark of cancer

- **Aberrant splicing** of oncogenes and tumor suppressors contributes to **PDAC progression.**
- **Spliceosome targeting** is a **novel, poorly understood** strategy in **PDAC**, unlike in **hematological cancers**.
- **Splicing modulation** links **genomic alterations** to functional tumor behavior — key for **precision oncology**.

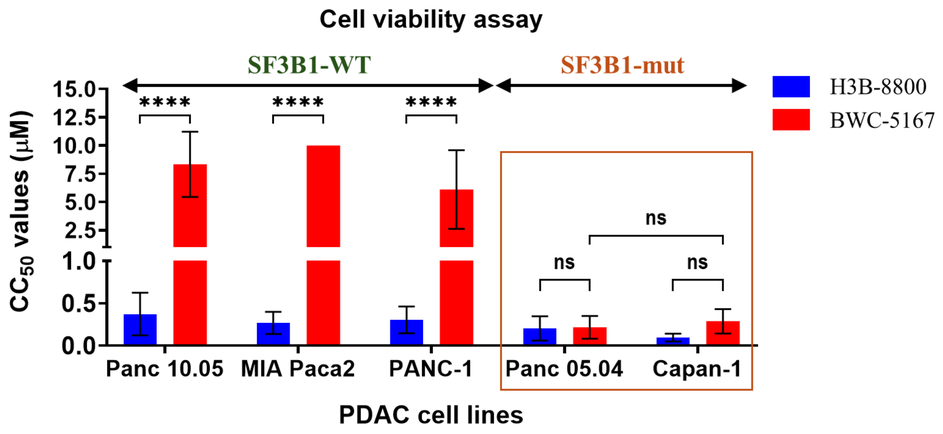
Genomic profile of the PDAC lines used in the study

	Panc 10.05	PANC-1	MiaPaCa-2	Capan-1	Panc 05.04	PDAC Frequency
KRAS	G12D (MS)	G12D (MS)	G12C (MS)	G12V (MS)	G12D (MS)	~ 95%
TP53	I255N (MS)	R273H, P72R (MS)	R248W (MS)	A159V (MS)	NR	~ 90%
CDKN2A	WT	HD	HD	HD	Del	~ 50-75%
SMAD4	NR	WT	WT	Mut/Del (Null)	NR	~ 50-60%
MTAP	WT	Del	Del	Del	Del	~ 15%
SF3B1	WT	WT	WT	FS	K700E (MS)	~ 2%

WT; Wild type
MS; Missense Mutations
FS; Frameshift Mutations
HD; Homozygous Deletion
Del; Deletion
NR; Not reported

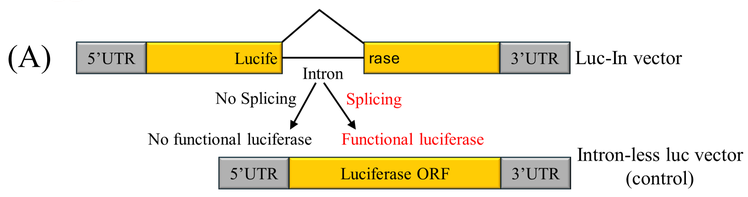
Results

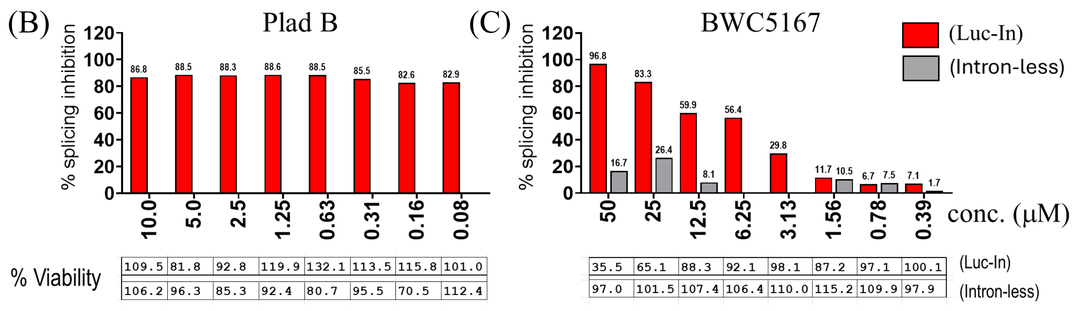
BWC5167 shows preferential cytotoxicity in SF3B1-mutant PDAC lines



Five genetically distinct PDAC cell lines were treated with BWC5167, H3B-8800 or DMSO control for 72 h. Cell viability was measured using the CellTiter-Glo luminescent assay (Promega). Data represents mean ± SD (****p<0.0001, 2way ANOVA)

BWC5167 triggers intron-retention in a luciferase-intron reporter system

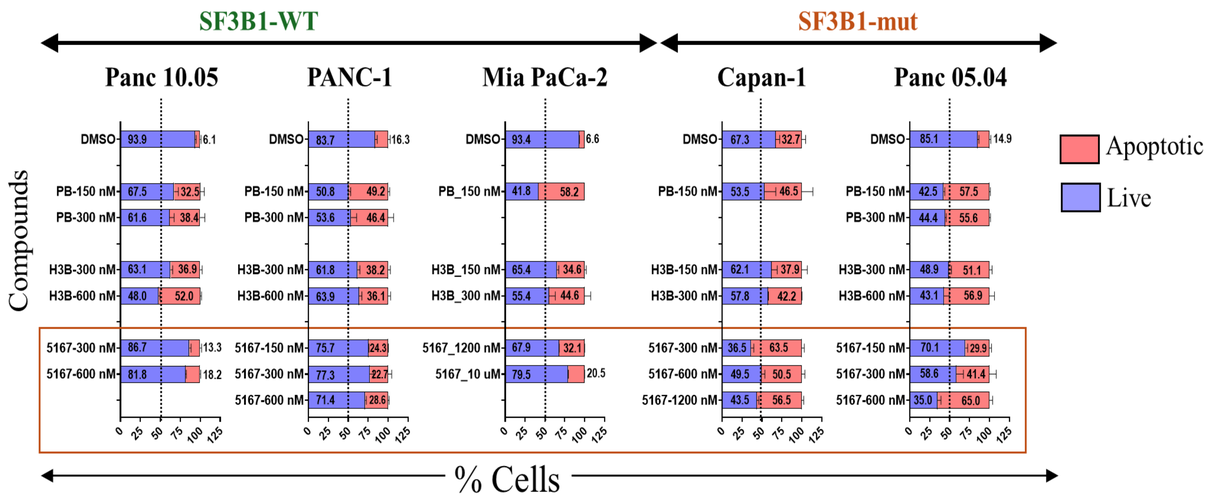




% Viability								
(Luc-In)	109.5	81.8	92.8	119.9	132.1	113.5	115.8	101.0
(Intron-less)	106.2	96.3	85.3	92.4	80.7	95.5	70.5	112.4

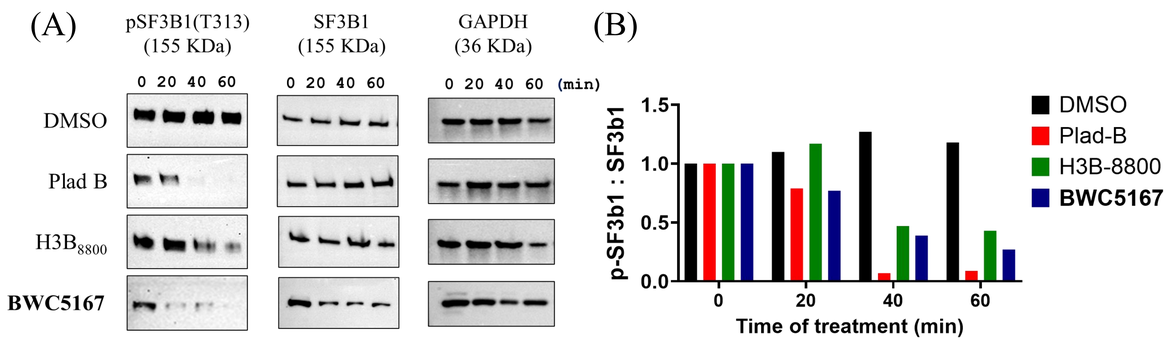
Selective inhibition of intron-containing luciferase reporter activity by BWC5167. (A) Schematic diagram of luciferase reporter constructs: intron-containing and intron-less control. (B) The luciferase-intron and intron-less reporter lines were subjected to (B) Pladienolide B and (C) BWC5167 treatment. 8h post-treatment luminescence was measured.

BWC5167 induces selective apoptosis in SF3B1-mutant cancer cell lines



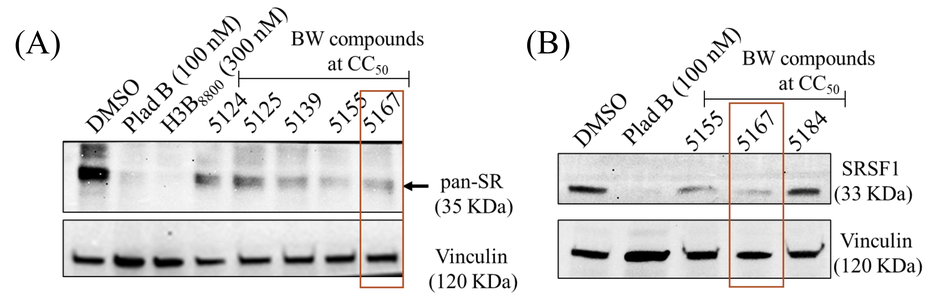
PDAC lines were treated with BWC5167, Pladienolide B, H3B-8800 or DMSO for 72 h. Apoptotic cells were quantified using Annexin V/7-AAD staining (BioLegend) and analyzed by flow cytometry (BD LSRFortessa) and BD FACSDiva™ software.

BWC5167 reduces phospho-SF3B1 levels in Panc 05.04 cells in a time-dependent manner



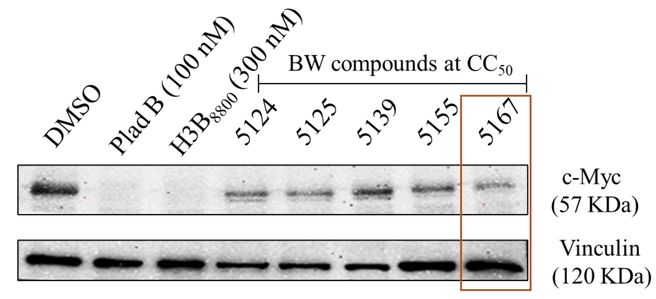
Panc 05.04 cells were treated with BWC5167, Pladienolide B, H3B-8800, or DMSO for the indicated time points. Protein lysates were immunoblotted for phospho-SF3B1 and total SF3B1, with GAPDH as the loading control. (A) Representative Western blots. (B) Quantification of phospho-SF3B1 levels relative to total SF3B1.

BWC5167 disrupts SR protein homeostasis in Panc 05.04



Panc 05.04 cells were treated with BW compounds, Pladienolide B, H3B-8800 or DMSO for 24 h. Protein lysates were immunoblotted for A) pan-SR proteins and B) SRSF1, Vinculin served as the loading control.

BWC5167 downregulates c-Myc levels in Panc 05.04



24h lysates from Panc 05.04 cells were immunoblotted for c-Myc. Vinculin served as the loading control.

Conclusion

1. BWC5167 selectively induces apoptosis in SF3B1-mutant PDAC cells via disruption of the splicing machinery, evidenced by intron retention, reduced phospho-SF3B1, and loss of SRSF1.
2. Unlike Pladienolide B and H3B-8800, which show broad toxicity, BWC5167 is selective to SF3B1 mutation.
3. c-Myc downregulation in Panc05.04 indicates suppression of oncogenic signaling.
4. Future work includes transcriptomic profiling, biomarker discovery, and in vivo validation.

References

1. Bradley & Anczukow, Nat Rev Cancer, 2023 (*PMID: 36627445*)
2. Alors-Pérez et al., Mol Cancer, 2021 (*PMID: 38790138*)
3. Seiler et al., Nat Med, 2018 (*PMID: 29457796*)
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** All the authors are employees of Bugworks Research India Pvt. Ltd.