BWC5077, a Potent and Novel Small-Molecule CDK7 Inhibitor suppresses transcriptional activity and arrest cell cycle through MYC

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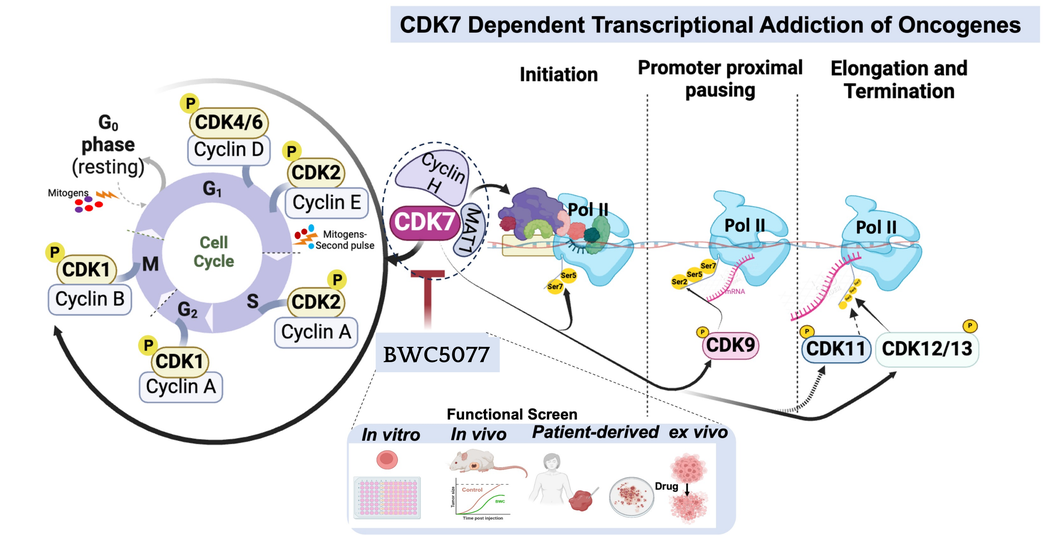
Introduction



- CDK7 (Cyclin-dependent kinases 7) is aberrantly overexpressed in various cancers
- It controls cancer cell proliferation by:
  - Regulating critical enzymes of the cell cycle (i.e. CDK1, CDK2, CDK4, CDK6)
  - Promoting transcription of oncogenes (c-MYC) through RNA Pol II phosphorylation
- Given its central role, CDK7 is an attractive target for oncotherapy

BWC5077 is a selective and potent CDK7 inhibitor

A

Target	BWC5077 Kd (nM)	Fold Selectivity (Kd CDKx/ Kd CDK7)
CDK7	**2.4**	**1**
CDK9	120	50
CDK2	240	100
CDK12	250	104
CDK13	900	375
CDK6	1700	708
CDK1	1900	791
CDK4	>10000	>4166

B

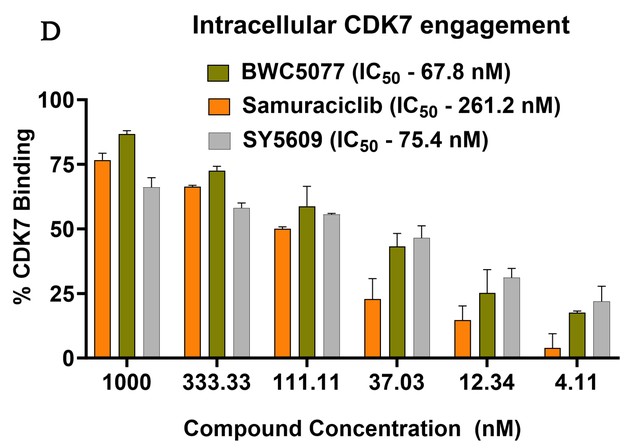
Compounds	Biochemical (Radiometric) IC_{50}
Samuraciclib	15 nM
SY5609	11 nM
BWC5077	**28 nM**

C

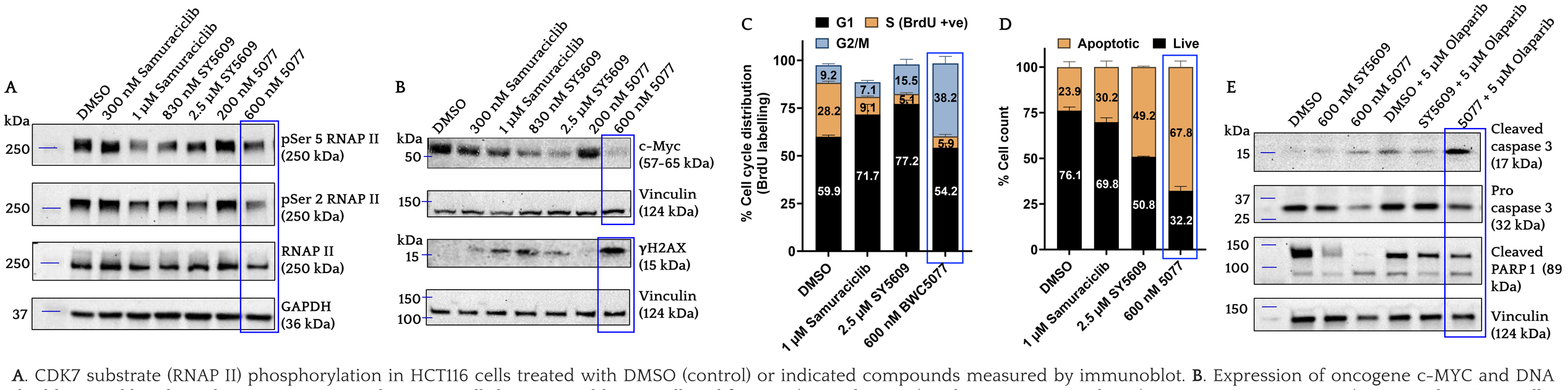
Compounds	HCT 116 CC_{50}
Samuraciclib	1 µM
SY5609	2.5 µM
BWC5077	**600 nM**

A. Selectivity profiling of BWC5077 using KINOMEscan™
B. Inhibition of CDK7 enzyme activity in a radiometric kinase assay
C. Cytotoxic concentration (CC_{50}) of BWC5077.
D. CDK7 cellular target engagement determined by NanoBRET assay

D



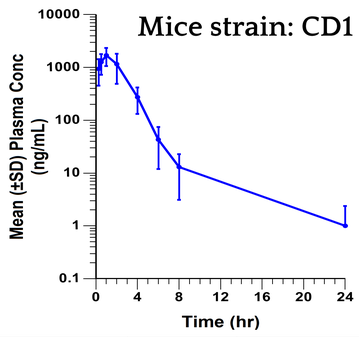
BWC5077 inhibits CDK7 substrate phosphorylation and c-Myc driven cell proliferation leading to G2/M arrest and tumour cell apoptosis



A. CDK7 substrate (RNAP II) phosphorylation in HCT116 cells treated with DMSO (control) or indicated compounds measured by immunoblot. **B**. Expression of oncogene c-MYC and DNA double strand break marker γH2AX in treated HCT 116 cells by immunoblot. **C.** Cell proliferation (C - BrdU assay) and **D,** apoptosis analysis (Annexin V/7AAD staining) in treated HCT 116 cells. **E**. Expression of apoptotic makers (cleaved caspase 3 and cleaved PARP1) in MDA-MB-231 (TNBC) cells treated with DMSO (control) or indicated compounds measured by immunoblot.

BWC5077 pharmacokinetics

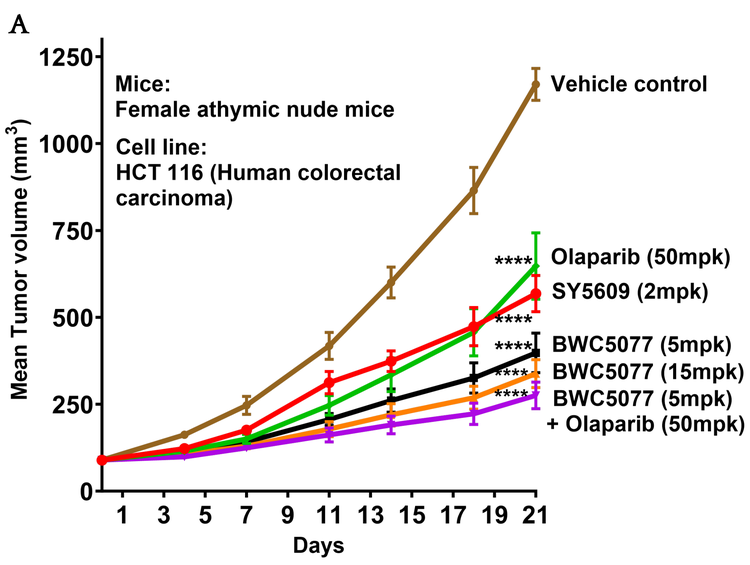
Route of administration: Oral (30 mpk)

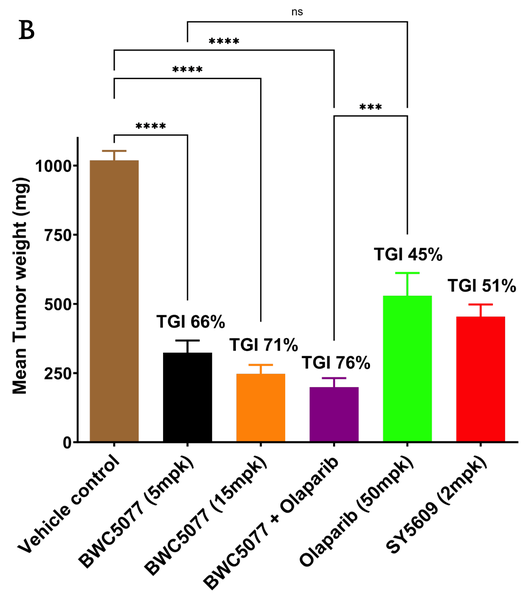


Parameters	Units	Estimate
T_{max}*	hr	1.0 (1.0-1.0)
C_{max}	ng/mL	1692 ± 631
AUC_{0-t}	hr.ng/mL	4094 ± 2031
AUC_{0-inf}	hr.ng/mL	4102 ± 2030
T 1/2	hr	0.88 ± 0.13
*Median (Min-Max)		

The dose to be used for the CRC xenograft model was determined to be 15mpk

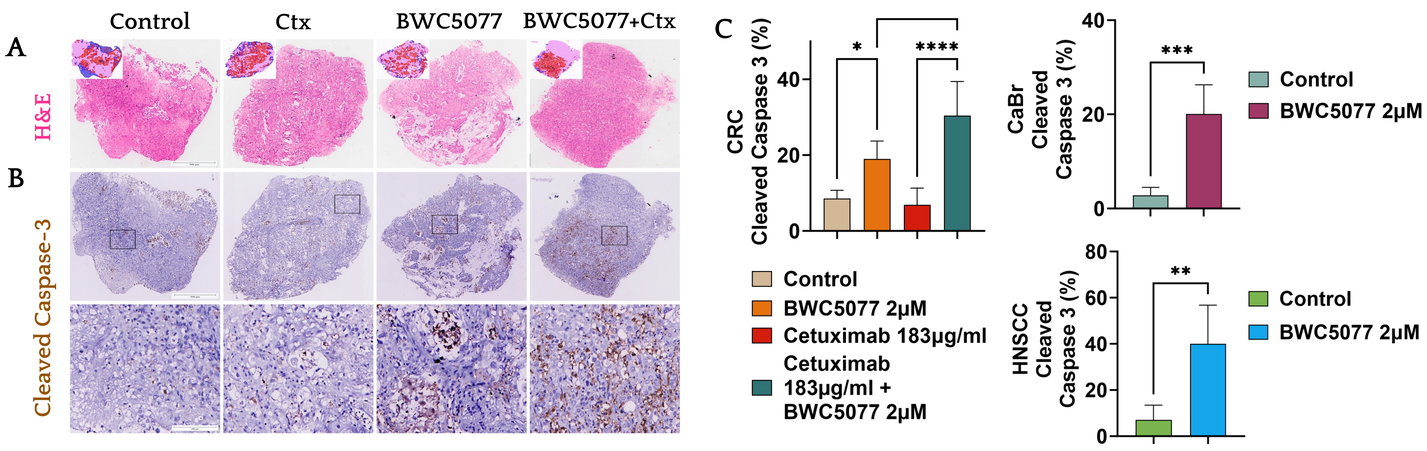
In a CRC xenograft model, BWC5077 alone or combined with Olaparib significantly reduced tumour growth





HCT 116 ($5X10^6$) cells were inoculated subcutaneously into female athymic nude mice. When tumour volume reached ≈ 100 mm^3, mice (N= 8 /group) were randomised and treated (as indicated in the legend) for 28 days. Values represent Mean ± SEM of 8 animals in each group. Two-way ANOVA followed by Bonferroni test. Average tumour volume (A) /weight (B) with SEM (****p < 0.0001). TGI - Tumour growth inhibition.

Ex vivo screening identifies BWC5077 as effective in different cancer types



A. Representative histological sections of colorectal cancer stained with H&E (Inset Digital AI mark-up, red - tumour, pink - stroma). **B.** Representative histological slices from (A), stained for apoptosis marker cleaved caspase 3, **C.** Expression levels of cleaved caspase 3 measured in tissue treated with BWC5077 in different cancer types, Scale bar 500 µm and 100 µm. Data represents SEM (****p < 0.0001, t-test)

Conclusions

- BWC5077 is a novel CDK7 inhibitor with potency of <50 nM and selectivity >100-fold over other CDKs
- Effectively inhibits CDK7 substrate phosphorylation at sub µM concentration and promotes G2/M arrest with DNA damage and leads to apoptosis
- Displays favourable oral bioavailability in CRC xenograft model.
- Demonstrates significant tumour growth inhibition alone or in combination with Olaparib in CRC xenograft model
- Effectively induces apoptosis in patient-derived tumour ex-vivo samples
- Patient-specific responses to synergistic therapy was identified using ex vivo organotypic cultures

[1] All the authors are employees of Bugworks Research India Pvt. Ltd.