81. Block Mitotic Progression for targeted therapy

(Northwestern University)

Asset Overview

Product Type	Others
Disease Area	Oncology
Indication	Tiple-negative breast cancer (TNBC)
Current Stage	Lead Optimization
Target	Cdc20
MoA	degrade cell division cycle protein 20, or Cdc20
Brief Description	• Blockade of mitotic progression is an ideal approach to induce mitotic catastrophe that sup- presses cancer cell expansion. Cdc20 is a critical mitotic factor governing anaphase initiation and the exit from mitosis through recruiting substrates to APC/C for degradation. • Inventors designed a proteolysis targeting chimera, called CP5V, which comprises a Cdc20 ligand and VHL binding moiety bridged by a PEG5 linker that induces Cdc20 degradation. Inventors characterized the effect of CP5V in destroying Cdc20, arresting mitosis, and inhibiting tumor progression by measuring protein degradation, 3D structure dynamics, cell cycle control, tumor cell killing and tumor inhibition using human breast cancer xenograft mouse model. • CP5V can specifically degrade Cdc20 by linking Cdc20 to the VHL/VBC complex for ubiquitination followed by proteasomal degradation. Induced degradation of Cdc20 by CP5V leads to significant inhibition of breast cancer cell proliferation and re-sensitization of Taxol-resistant cell lines. Results based on a human breast cancer xenograft mouse model show a significant role for CP5V in suppressing breast tumor progression. • CP5V-mediated degradation of Cdc20 could be an effective therapeutic strategy for anti- mitotic therapy.
Intellectual Property	US20220241424A1
Publication	A novel strategy to block mitotic progression for targeted therapy. EBioMedicine. (2019)
Inventors	Yong Wan, Zhuan Zhou, Junlong Chi, Gary Schiltz

Highlights

- In in vitro and in vivo models of TNBC, the researchers observed induced destruction of Cdc20 by their novel compound that led to significant inhibition of cancer cell proliferation.
- Xenograft model studies in mice demonstrated a 70% decrease in size and weight of the tumor compared to the placebo, with no significant toxicity observed.
- When taxane-resistant cancer cells were treated with a combination of the Cdc20 PROTAC and paclitaxel, a taxane that is a common chemotherapeutic agent for TNBC, the cells displayed a re-sensitization and cytotoxic response to the paclitaxel.
- Targeting Cdc20 for degradation can be an effective therapeutic strategy for anti-mitotic breast cancer therapy or combination therapy for drug resistance.

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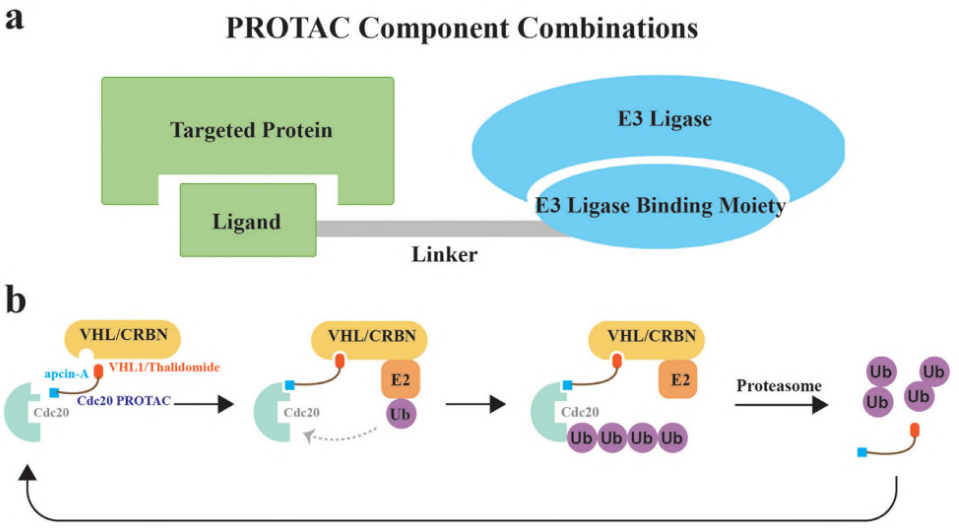
Key Data

Design and selection of Cdc20 PROTACs



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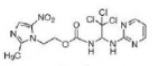
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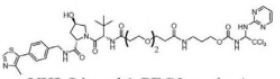
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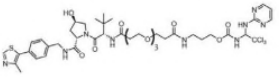
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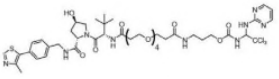
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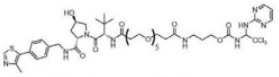
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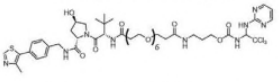
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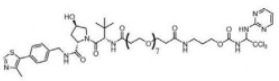
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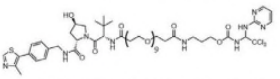
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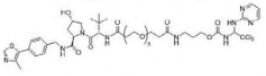
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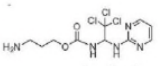
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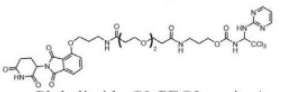
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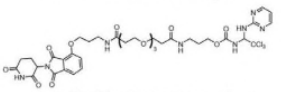
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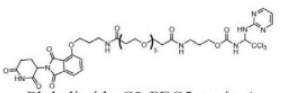
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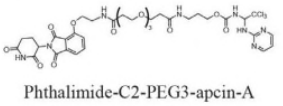
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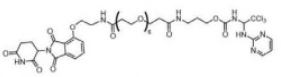
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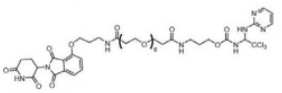
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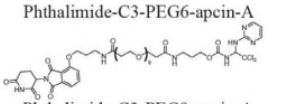
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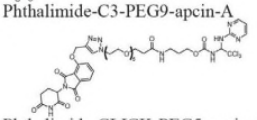
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To be continued

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(a) The mechanism of PROTAC technology is to recruit an endogenous ubiquitin protein ligase in order to induce the ubiquitination of targeted proteins for its degradation. Bifunctional PROTAC molecules bind to the targeting protein with one end while the other end binds an E3 ligase to form a ternary complex. The recruited E3 ligase then mediates the transfer of ubiquitin from an E2 enzyme to the targeting protein. The ternary complex dissociates, and the ubiquitinated targeting protein is removed by the proteasome.
b) The Flowchart of Cdc20 PROTACs mechanism. Apcin-A is utilized as Cdc20 targeting ligand, and VHL and CRBN binding moieties VHL1 and thalidomide are respectively used to recruit the VHL/VBC complex and Celebron E3 ligase in the Cdc20 PROTACs. A series of polyethylene glycol (PEG) molecules were used to link apcin-A and VHL1/thalidomide.
c) The structure of apcin, apcin-A and series of Cdc20 PROTACs designed in the present study.

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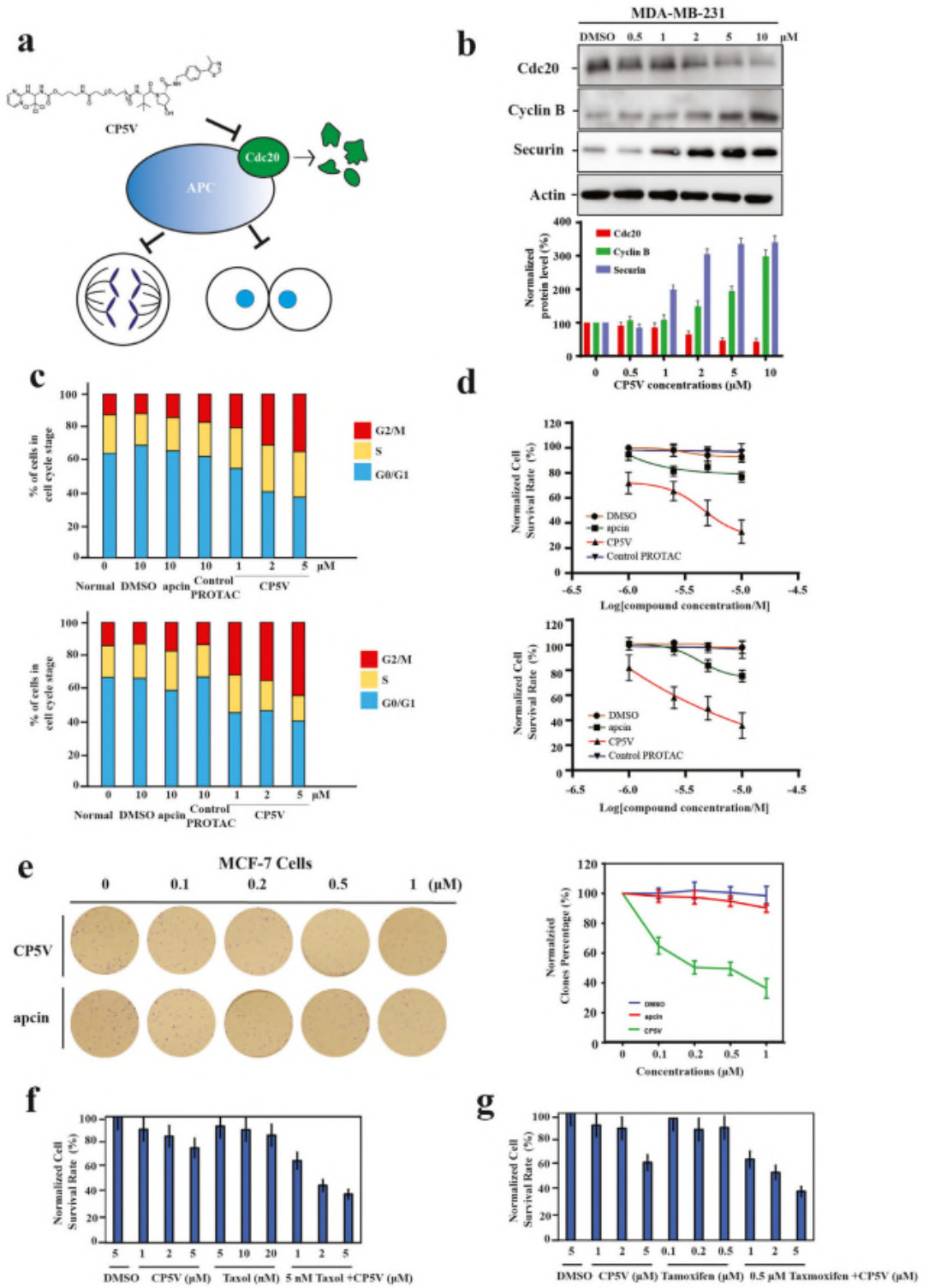
CP5V significantly inhibits mitotic progression, cancer cell growth, and further induces cancer cell death.



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CP5V significantly inhibits mitotic progression, cancer cell growth, and further induces cancer cell death.

(**a**) Schematic description of the process of targeting Cdc20 for degradation by CP5V leading to inhibition of chromatid separation and the exit from mitosis. (**b**) CP5V-mediated Cdc20 degradation results in accumulation of cyclin B. MDA-MB-231 cells were treated with CP5V at the indicated dose for 10 h. Cdc20 and cyclin B levels were then determined by Western blotting. The Western Blot is representative of 3 independent experiments (n = 3). Data are mean ±SEM. (**c**) CP5V induces mitotic arrest. The MDA-MB-231 (upper panel) and MDA-MB-435 cells (lower panel) were initially synchronized by double-thymidine treatment followed by a 9-hour release with fresh medium and indicated treatments for 16 h. Cell cycle profile was then measured by flow cytometry and analyzed by ModFit LT. (**d**) CP5V causes significant inhibition of cell growth in MDA-MB-231 (upper panel) and MDA-MB-435 cells (lower panel). MDA-MB-231 and MDA-MB-435 cells were plated in 96-well plates at the concentration of 30 0 0 cells/well and treated with DMSO, apcin, Control CP5V and CP5V for 72 h and the cell survival activity was measured by CCK8 assay. The IC50 of CP5V for MDA-MB-231 cells is 2.6 μM and 1.99 μM for MDA-MB-435 cells. The test was performed in triplicate (n = 3). Data are mean ±SEM. (**e**) Clonogenic assay of the effect of CP5V on MCF7 cells. 200 MCF7 cells were plated in 6-well plate and treated with CP5V at gradient concentrations (0.1, 0.2, 0.5, 1 μM) for 24 h following culture for 2 weeks. To quantify the colony formation, the cells were stained with crystal violet and the numbers of colonies were counted and quantified by Image J. Left panel shows the representative clones. Right panel shows the statistical results. Data are presented as the mean ±standard error of the mean (S.E.) of three independent samples ($**p$ -value < 0.01). (**f**) CP5V can restore the Taxol-induced cytotoxic response for Taxol-resistant MDA-MB-435 eb. cells and can inhibit their growth. (**g**) CP5V rescues the endocrine response for tamoxifen-resistant MCF-7 4HTR cells. The measurements were performed in triplicate (n = 3). Data are mean ±SEM.

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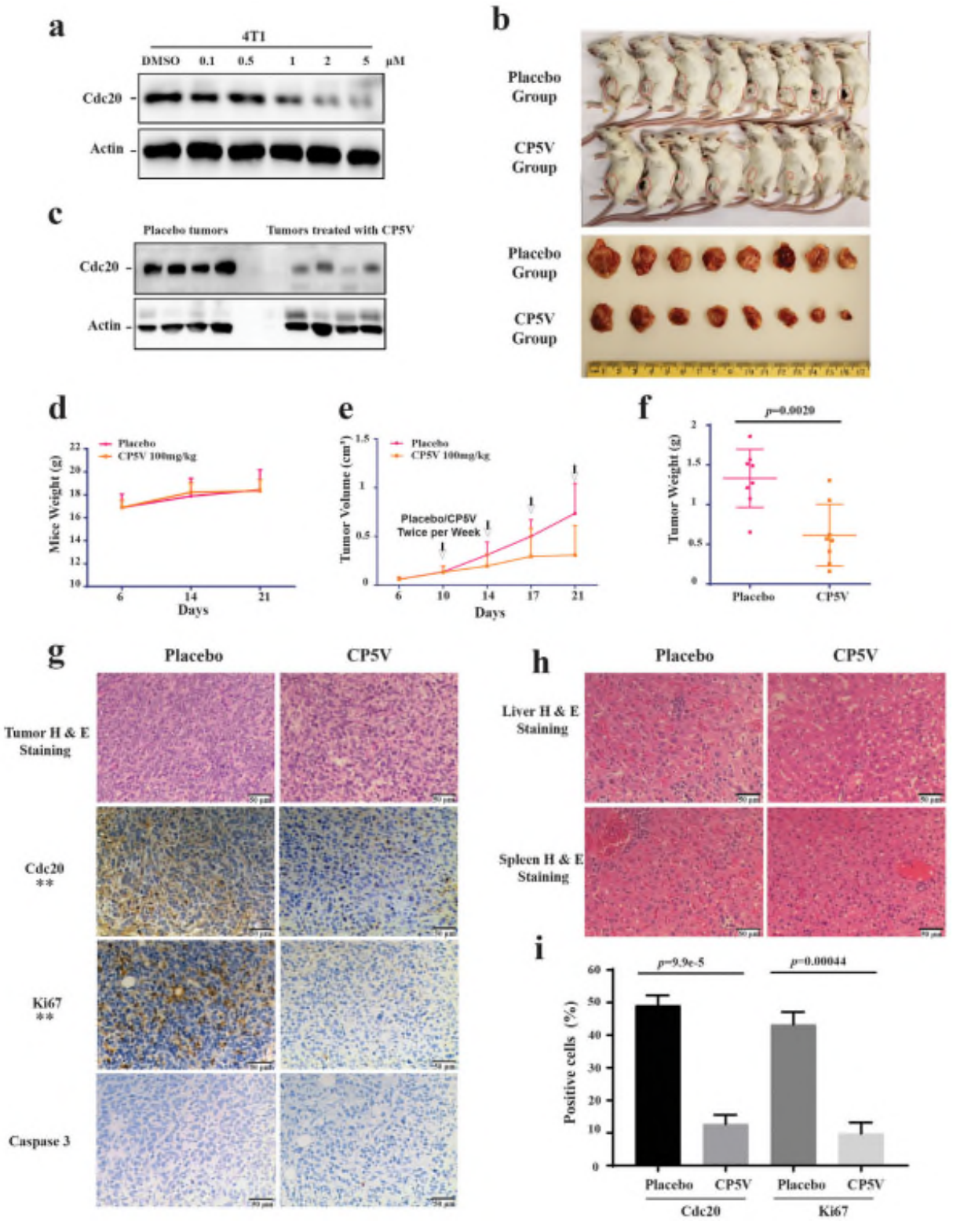
CP5V is a potent inhibitor that suppresses breast tumor progression with no toxicity in the 4T1 xenograft model.



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CP5V is a potent inhibitor that suppresses breast tumor progression with no toxicity in the 4T1 xenograft model.

(**a**) CP5V can efficiently cause degradation of Cdc20 in 4T1 cells lines *in vitro* . (**b** - **e**) CP5V dramatically inhibits tumor growth. 4T1 cells were implanted into the mammary fat pad of BALB/c mice. Drug treatment started on the 10th day. Placebo or CP5V at the dose of 100 mg/kg was administrated twice a week for two weeks. (b) The image of 4T1 xenograft tumors harvested after 21 days. (**c**) Western blotting assays the expression of Cdc20 in 4T1 xenograft tumor. (**d**) Body weight curve of mice. (**e**) Tumor growth curve. Tumor volume was measured twice a week. The asterisk represents the significant difference (p -value < 0.05) between group Placebo and group CP5V. (**f**) Tumor weight curve. (**g**) Immunohistochemistry Staining of H & E, Cdc20, Ki67, and activated caspase 3 in 4T1 xenograft tumors. Scale bar, 50 μm. (**h**) H & E staining of tumor and liver of mice from both Placebo and CP5V group. (**i**) Quantification of Ki67 and activated caspase 3 positive cells in 4T1 xenograft tumors. Data are mean ±SEM.