CDK2 Heterobifunctional Degraders Co-Degrade CDK2 and Cyclin E Resulting in Efficacy in CCNE1-Amplified and Overexpressed Cancers

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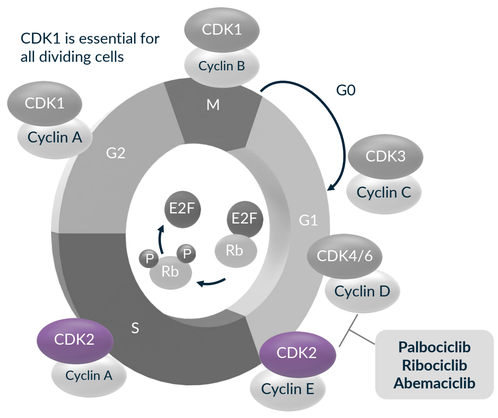
INTRODUCTION

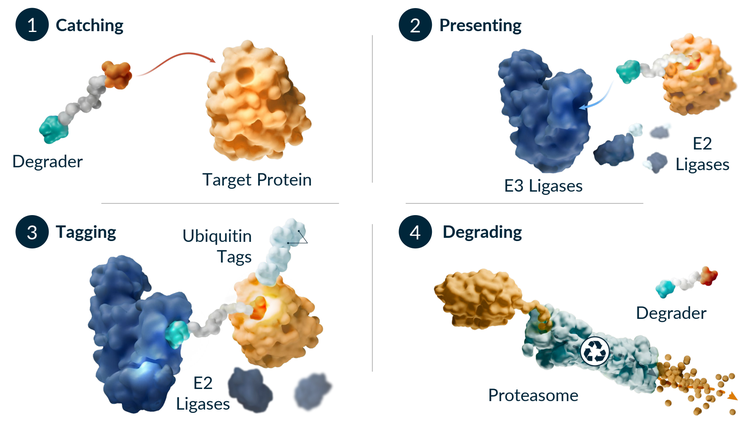
CCNE1 amplification (*CCNE1*^{amp}) is a common genetic alteration in cancer driving high cyclin E1 expression and aberrant CDK2-cyclin E1 activity.[1] Despite activity of CDK2 small-molecule inhibitors (SMI), their therapeutic margins are limited by poor CDK selectivity, including CDK1 which is known to cause both heme and GI DLTs.[2,3] We developed CDK2 degraders with high selectivity for CDK2 over CDK1 and other CDKs that also unexpectedly led to cyclin E1 degradation and potent and complete suppression of RB phosphorylation at concentrations with negligible CDK1 degradation. The improved potency and selectivity of the degrader for CDK2 drives antiproliferative activity with greater specificity for *CCNE1*^{amp} cancer cells relative to the SMIs. Additionally, depletion of CDK2 and cyclin E1 re-sensitized Palbociclib-adapted (PA) breast cancer cells to cell cycle arrest. Using an orally administered degrader we demonstrate deep and sustained RB pathway suppression, which is needed to induce stasis in *CCNE1*^{amp} tumors. These results highlight the potential of this modality to target CDK2 potently and selectively in this biomarker-defined patient population with high unmet need.

CDK2 Biology and Target Rationale



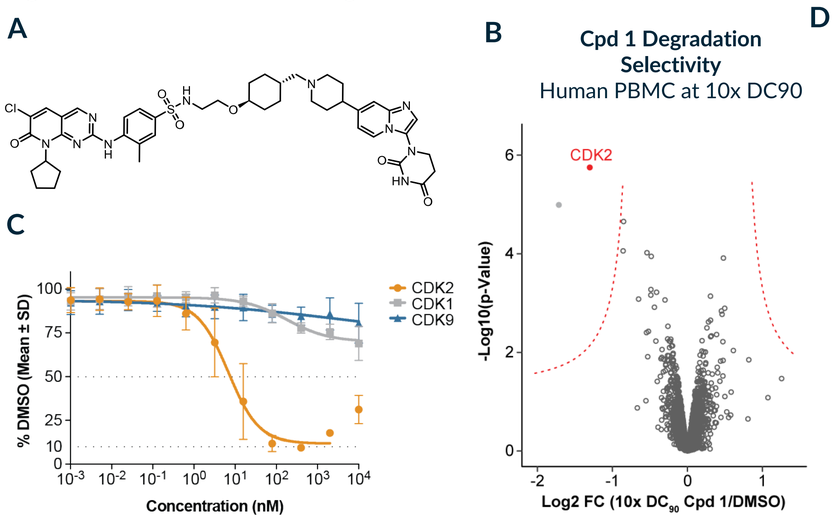
- CDK2 is a master regulator of G1-S cell cycle pathway[1]
- *CCNE1*^{amp} serves as a biomarker of CDK2 dependence across cancer types[4]
- *CCNE1*^{AMP} occurs frequently in ovarian and endometrial cancers and is resistant to SoC therapies[5-7]
- High CCNE1 expression associated with resistance to CDK4/6 inhibitors and hormone therapy in HR+/HER2- advanced BC.[8]
- CDK2 SMI have been hindered by high kinase homology with CDK1 (and other CDKs) and poor selectivity[2]

Proteome Editing with Targeted Protein Degradation



RESULTS

Figure 1. Cpd 1 achieves degradation selectivity for CDK2

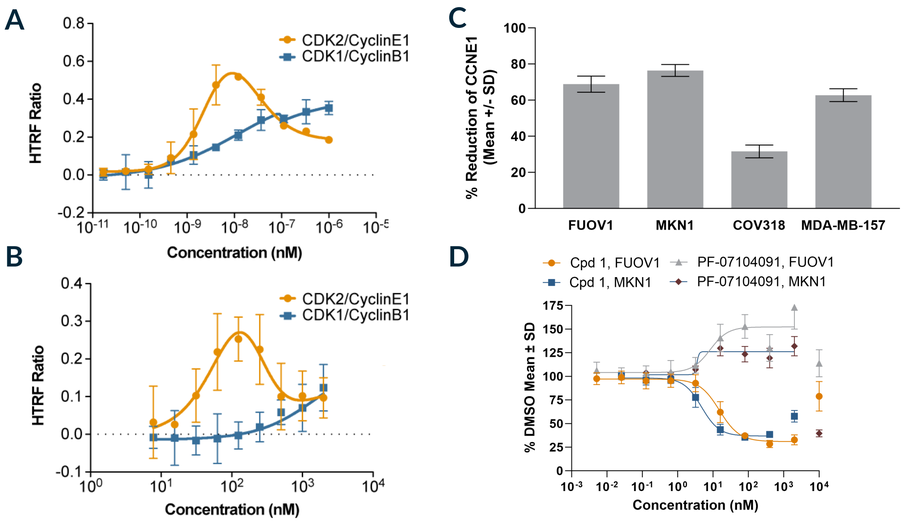


D

	Cpd 1	Cpd 2
CDK2 Potency, absDC50 (nM) / Dmax (%)		
MKN1	11 / 95%	9 / 95%
HCC1569	10 / 94%	22 / 92%
RB Pathway Modulation, pRB absIC50 (nM) / Imax(%)		
MKN1	27 /96%	7 / 95%
HCC1569	34 / 87%	21 / 86%
Anti-proliferation activity, rel IC50 (nM)		
MKN1 CCNE1^{amp}	7	17
HCC1569 CCNE1^{amp}	16	23
OVCAR3 CCNE1^{amp}	28	109
TOV21G CCNE1$^{non\text{-}amp}$	4,000	6,000
Selectivity (TOV21G cells)		
CDK2 absDC50 (nM)	8	15
CDK1 fold-sparing (by absDC50)	>1250	243
CDK9 fold-sparing (by absDC50)	>1250	>670

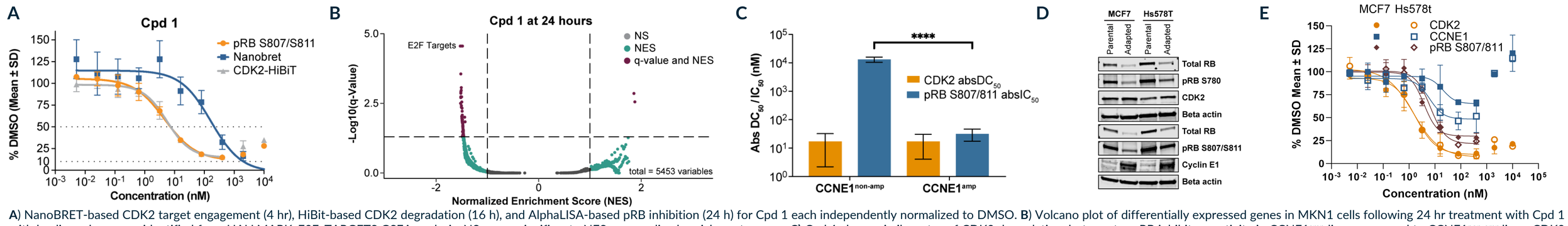
A) Chemical structure of the CRBN-based CDK2 degrader, Cpd 1. **B**) CDK2 selectivity proteomics in human PBMCs following 24 hr treatment with Cpd 1. Gray filled in circle is PIP4K2C, the only other significantly downregulated protein. **C)** CDK degradation selectivity in TOV21G with Cpd 1. **D)** Summary of potency and selectivity data for Cpd 1 and Cpd 2. Absolute (abs) and relative (rel) values are indicated.

Figure 2: Selective CDK2-cyclin E1 degradation results from a preferred ternary complex formation w/ CDK2-cycE1 vs CDK1-cycB1



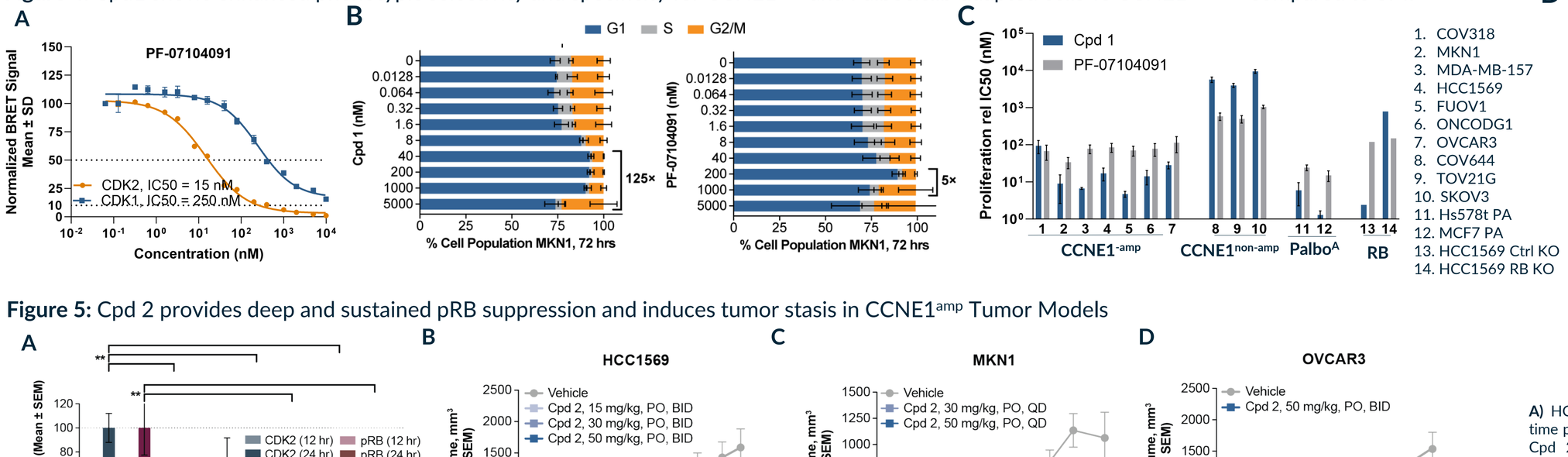
Cpd 1 shows robust TCF **(A)** and *in vitro* ubiquitination **(B)** with CDK2-cyclin E1 compared to CDK1-cyclin B1 by HTRF. **C)** Cpd 1 co-degrades cyclin E1 in the cell types indicated following 24 hr treatment with Cpd 1. **D)** Cpd 1 decreases cyclin E1 protein levels, in contrast to PF-07104091 which upregulates CCNE1 protein levels, after 24 hr treatment.

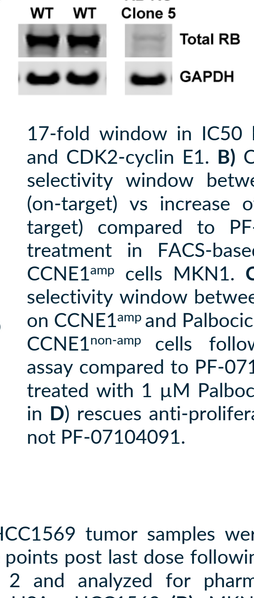
Figure 3. Cpd1 inhibits RB-E2F pathway in CCNE1^{amp} cancer cell lines and Palbociclib-adapted (PA) breast cancer cells[9]



A) NanoBRET-based CDK2 target engagement (4 hr), HiBit-based CDK2 degradation (16 h), and AlphaLISA-based pRB inhibition (24 h) for Cpd 1 each independently normalized to DMSO. **B**) Volcano plot of differentially expressed genes in MKN1 cells following 24 hr treatment with Cpd 1 with leading edge genes identified from HALLMARK_E2F_TARGETS GSEA analysis. NS = non-significant. NES = normalized enrichment score. **C)** Cpd 1 shows similar rates of CDK2 degradation, but greater pRB inhibitory activity in CCNE1^{amp} lines compared to CCNE1$^{non\text{-}amp}$ lines. CDK2 absolute DC50 (abs DC50) and pRB absIC50 were determined after 14 and 24 hr treatment by AlphaLISA, respectively. **D)** Cyclin E1 is upregulated and total RB is downregulated in Palbociclib-adapted (PA) MCF7 and Hs578t cells relative to parental controls. **E)** Cpd 1 co-degrades CDK2 and cyclin E1 in MCF7 and Hs578t PA breast cancer cells after 24 hr co-treatment with 1 µM Palbociclib.

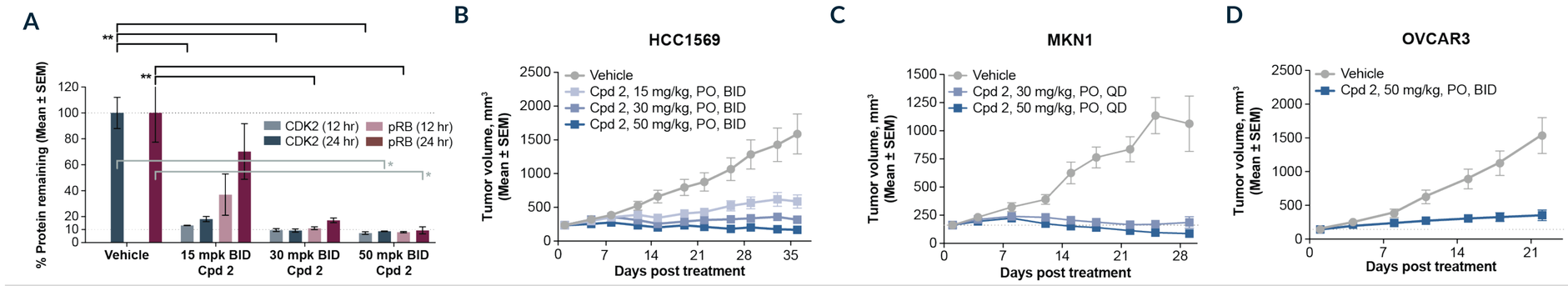
Figure 4. Cpd1 shows enhanced phenotypic selectivity and specificity for CCNE1^{amp} and Palbociclib-adapted cells vs CCNE1$^{non\text{-}amp}$ compared to SMI





A) NanoBRET target engagement assay of CDK1-cyclin B1 and CDK2-cyclin E1 in 293 cells with PF-07104091 shows 17-fold window in IC50 between CDK1-cyclin B1 and CDK2-cyclin E1. **B)** Cpd 1 shows an improved selectivity window between maximum G1 arrest (on-target) vs increase of G2/M population (off-target) compared to PF-07104091 after 3-days treatment in FACS-based cell cycle readout in CCNE1^{amp} cells MKN1. **C)** Cpd 1 shows superior selectivity window between antiproliferative effects on CCNE1^{amp} and Palbociclib-adapted cancer cells vs CCNE1$^{non\text{-}amp}$ cells following 7-day proliferation assay compared to PF-07104091. PA cells were co-treated with 1 µM Palbociclib. RB KO (WB shown in **D**) rescues anti-proliferative effect of Cpd 1, but not PF-07104091.

Figure 5: Cpd 2 provides deep and sustained pRB suppression and induces tumor stasis in CCNE1^{amp} Tumor Models



A) HCC1569 tumor samples were collected at indicated time points post last dose following 3 days BID dosing w/ Cpd 2 and analyzed for pharmacodynamic activity by AlphaLISA. HCC1569 **(B)**, MKN1 **(C)**, and OVCAR3 **(D)** CCNE1^{amp} subcutaneous tumors were established in immunocompromised mice. Cpd 2 was dosed PO as indicated. Tumor volume estimated by caliper measurements..

METHODS

***In vitro* TCF and Ubiquitination Analyses**

Homogeneous Time-Resolved Fluorescence (HTRF) assay was conducted to evaluate the formation of TC involving CDK2/cyclinE1 and CDK1/cyclinB1 with CRBN/DDB1 in the presence of Cpd 1. An HTRF assay was used to measure the ubiquitination of CDK2/CyclinE1 and CDK1/CyclinB1 with the E1,E2,E3 enzyme complex in the presence of Cpd 1.

***In vitro* and *In vivo* Experiments**

For *in vitro* degradation assays in TOV21G, cells were treated with Cpd for 24 h and total CDK1/2/9 protein assessed by AlphaLISA. For all other cell lines, Cpd was treated for 14 hrs for CDK2 AlphaLISA, 24 hrs for pRB S807/811 and CCNE1 AlphaLISA, or 7 days for Cyquant proliferation assay. For 7-day Cyquant assay in MCF7 and Hs578t Palbociclib-adapted cells, Cpd 1 was treated in dose response with 1 µM Palbociclib. *In vivo* experimental details in figure captions.

Cell Cycle

MKN1 cells were treated with Cpd1, Cpd 2, or PF-07104091 in dose-response for 3 days. DNA replication and content were measured with Click-iT™ Plus EdU Alexa Fluor™ 647 Flow Cytometry Assay Kit and FxCycle™ PI/RNAse staining respectively and analyzed by flow cytometry.

Global Proteomic Selectivity Analysis

Human PBMCs treated with Cpd 1 for 24 hrs at 10x DC90 (500 nM) concentration. Tandem Mass Tag (TMT) discovery proteomics was performed. Statistical analysis was carried out using the Limma statistical package.

Transcriptomic Analysis

MKN1 cells treated with 80nM of Cpd 1 or DMSO for 24h were submitted for RNA sequencing. RNAseq analysis was performed using Kallisto and Sleuth. Pathway enrichment was determined by GSEA.

CONCLUSIONS

CDK2 degraders demonstrate antitumor activity in CCNE1^{amp} human xenograft tumor models after oral dosing. Our data provides molecular mechanistic underpinnings behind their potent and selective degradation of CDK2 and inhibition of downstream E2F signaling.

- CDK2 degraders achieve selective CDK2 degradation over closely-related CDKs by preferential ternary complex formation and ubiquitination. Cyclin E1 is co-degraded with CDK2.
- CDK2 degradation results in reduced RB phosphorylation and proliferative capacity in both CCNE1^{amp} cancer cells and breast cancer cells that have adapted to Palbociclib via upregulation of CCNE1 and reduction of RB expression, identifying biomarker-driven activity.
- CDK2 degraders offer several advantages over SMI: (1) catalytic CDK2 degradation to drive deep and sustained RB pathway suppression, (2) downregulation opposed to upregulation of CCNE1, and (3) phenotypic selectivity owing to enhanced CDK1 selectivity.
- Cpd 2, an orally bioavailable degrader, induces robust tumor stasis in numerous CCNE1^{amp} tumor models correlating with ≥90% sustained pRB inhibition
- These results highlight the potential for TPD to target CDK2 potently and selectively in CCNE1^{amp} cancers, an area of high unmet need

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DISCLOSURES

N.K., Z.S., K.Y., T.L., P.N.C., X.Z., K.L., M.S., C.L.H., D.C., S.B.B., K.S., R.M., H.J., J.D.G., M.W., and J.W. are employees of Kymera Therapeutics, Inc. and may hold stock or stock options. A.Y., A.P., L.S., and R.A. were employees of Kymera at the time of the study and may hold stock or stock options. P.N.C., X.Z., R.A., and M.W. are inventors on Kymera Therapeutics, Inc. patents.