Discovery of potent and selective CDK2 molecular glue degraders for the treatment of HR+/HER2- breast cancer, and CCNE1 amplified tumors

Nasrin Rastgoo, Leenus Martin, Jean-Francois Brazeau, Quinn Spalding, Gabrielle Blanco, Kyohei Hayashi, Susan Song, Jianguo Ma, Shu You, Alex Campos, Julia Toth, Jay Chung, Farhana Barmare, Hongfeng Gao, Meg McCarrick, Kevin Freeman-Cook and Peggy A. Thompson

#A026

Plexium, San Diego, CA

Nasrin.Rastgoo@plexium.com

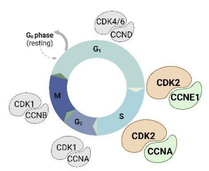
Abstract

The Cyclin-Dependent Kinase 2 (CDK2) is a key oncology target and promotes cell cycle progression by binding to its cyclin binding partners, especially, Cyclin E (CCNE1). The activated CCNE1/CDK2 complex phosphorylates RB and promotes cellular proliferation. CCNE1 amplification/overexpression drives aberrant cell cycle progression, and is associated with poor prognosis in multiple cancers, including ovarian and breast cancers. Activation of CDK2 by amplified CCNE1 is a key resistance mechanism in CDK4/6 inhibitor HR$^+$/HER2$^-$ metastatic breast cancer therapy. Additionally, CCNE1/CDK2 activation has also been associated with poor prognosis in ovarian and endometrial cancers. Current CDK2 inhibitors are limited by their diminished selectivity at efficacious exposures due to high similarity in the ATP-binding sites of other CDKs. Molecular glue degraders of CDK2 offer an attractive alternative strategy to achieve selective downregulation of CDK2 providing potential for improved clinical benefit. Plexium's integrated monovalent degrader platform resulted in the identification of novel, potent and selective CRBN-based CDK2 molecular glue (MG) degraders. Cryo-EM structures of the CRBN:MG:CDK2 ternary complex revealed a unique degradation complex that led to the design of multiple novel MG chemical series. Degradation potency was profiled using a HiBiT CDK2 cell line demonstrating dose dependent and proteasome mediated degradation. Dependency on CRBN was confirmed using a knock-out cell line; binding potency to CRBN also correlated with CDK2 degradation DC50. Potency and stability of CRBN:MG:CDK2 ternary complex formation corresponded with CDK2 degradation in both DC_{50} and D_{max}. Global proteomics demonstrated that these molecules were highly selective CDK2 degraders, without modulation of other CDKs or known CRBN neo-substrates and resulted in downregulation of E2F target proteins. Selective CDK2 degraders demonstrated potent inhibition of RB phosphorylation, cell cycle arrest and antiproliferative activity in CCNE1 amplified vs non-amplified cancer cell lines. In addition, treatment with CDK2 MGs counteracts resistance to CDK4/6 inhibition in HR+ breast cancer models. Oral administration of CDK2 degraders demonstrate *in vitro-in vivo* PK/PD correlation and anti-tumor activity in CCNE1 amplified mouse xenograft models. This supports the potential for CDK2 molecular glue degraders to treat CDK4/6 inhibitor-naïve and -resistant HR$^+$/HER2$^-$ breast cancer, and CCNE1 amplified patient populations.

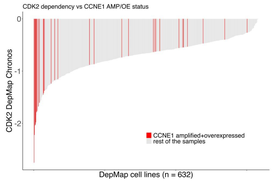
Introduction

- CDK2 associates with cyclin E (CCNE1) to regulate cell cycle progression. Aberrant Cyclin E levels activate CDK2 and correlate with poor survival in ovarian cancer patients
- Tumor cell lines with CCNE1 amplifications are sensitive to the loss of CDK2
- CDK2 degraders have the potential for superiority over inhibitors by (1) selectivity degrading CDK2 without modulating other CDKs and (2) disrupting CDK2 complexes
- Human cancers with cyclin E overexpression/amplification may be sensitive to loss of CDK2. ER$^+$/HER2$^-$ breast cancer patients treated with CDK4/6 inhibitors develop resistance which can be driven by cyclin E overexpression

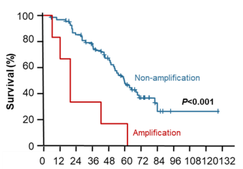
CDK2 Regulates G1 to S Cell Cycle Transition



Tumor Cell Lines with CCNE1^{amp} are Dependent on CDK2



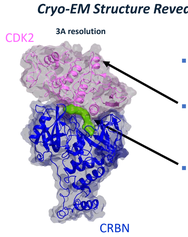
CCNE1^{amp} Correlates with Poor Survival (ovarian)



	Tumor Type	CCNE1^{amp} Frequency*
	Uterine	40%
	Ovarian	19%
	Gastric	11%
	Esophageal	8%
	Endometrial	8%
	Sarcoma	8%
	Bladder	6%
	NSCLC	5%

*cBioPortal frequency of CCNE1 amplification

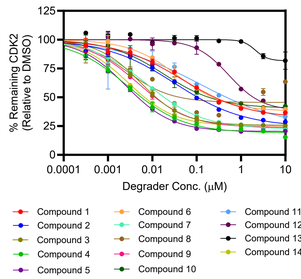
Cryo-EM Structure Reveals a Unique Degradation Complex and Opportunity for Superior Selectivity



- Structure-Based Drug Design led to the discovery of several novel scaffolds leading to high CDK2 selectivity
- High sequence homology of ATP binding pockets for CDK2 vs. CDK1 provides challenges to get CDK2 selectivity over CDK1 for current inhibitors
- CDK2/MGDs/CRBN complex engages a unique region of CDK2
  - Differs from current ATP-competitive inhibitors
  - Provides superior selectivity for CDK2
  - Differs from the conventional G-loop mechanism of action for traditional neosubstrates (such as IKZs, CK1α, WIZ and GSPT1)

Discovery of potent cereblon-based CDK2 molecular glue degraders

CDK2 Degradation in HEK293 HiBiT Cell Line



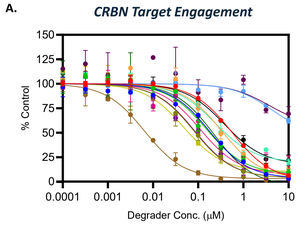
Summary of DC_{50}

Compounds	CDK2 Degradation	
	DC_{50} (nM)	D_{max} (%)
Compound 1	57	63
Compound 2	43	65
Compound 3	2	74
Compound 4	4	79
Compound 5	2	78
Compound 6	40	60
Compound 7	7	78
Compound 8	9	66
Compound 9	4	77
Compound 10	21	59
Compound 11	110	67
Compound 12	532	60
Compound 13	270	17
Compound 14	3	75

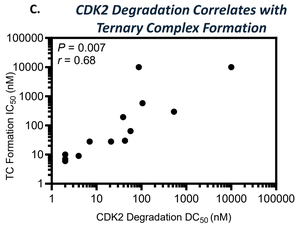
Figure 1. Profiling of CDK2 molecular glue degraders in HEK293 CDK2 HiBiT cell line. CDK2 HiBiT cells were treated with indicated compounds for 24h and the degradation of CDK2 was measured using a HiBiT assay (Promega). DC_{50} and maximum degradation (D_{max}) are indicated in the table.

Mechanism of CDK2 molecular glue degraders

CRBN Target Engagement



CDK2 Degradation Correlates with Ternary Complex Formation



Ternary Complex Formation

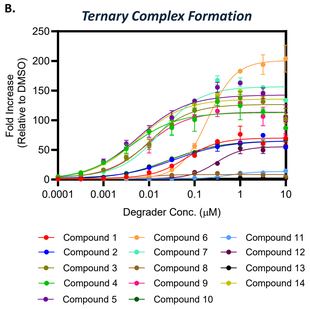
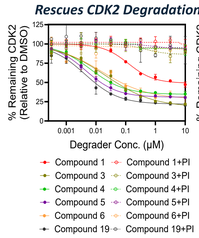
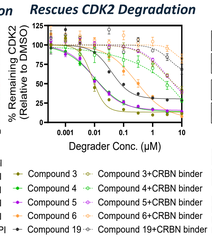


Figure 2. CDK2 molecular glues induce CRBN-CDK2 ternary complex (A) Target engagement of cereblon (CRBN) in HEK293 cells was quantitated using the NanoBRET™ Target Engagement assay platform (Promega). **(B)** Ternary complex of CDK2 and cereblon (CRBN)/DDBI in HEK293 cells was quantitated using a NanoBiT Protein:Protein Interaction Ternary Complex Assay (Promega). **(C)** Correlation of CDK2 degradation DC_{50} (HiBiT HEK293 cell line) and ternary complex formation IC_{50} for 14 CDK2 molecular glue degraders. The correlation coefficient (*r*) and *P* value are indicated in the graph.

Proteasome Inhibition Rescues CDK2 Degradation



CRBN Binding Displacement Rescues CDK2 Degradation



CRBN KO Rescues CDK2 Degradation

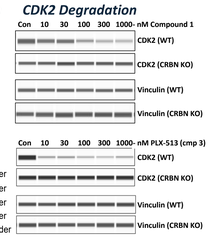


Figure 3. CDK2 degradation is cereblon and proteasome dependent. (A) CDK2 HiBiT HEK293 cells were treated ± proteasome inhibitor (0.1 μM bortezomib) for 1h, followed by 6h treatment with varying concentrations of different CDK2 degraders **(B)** MKN1 cells were treated with different CDK2 degraders ± a potent CRBN binder (10 μM) for 24h and CDK2 protein level was measured by AlphaLISA assay **(C)** CDK2 protein levels after treating with increasing concentrations of different CDK2 degraders for 24h in CRBN WT and KO Jurkat cell lines. CDK2 protein was detected using a Jess Simple Western (Protein Simple).

Selective CDK2 degraders modulate E2F target genes in global proteomics

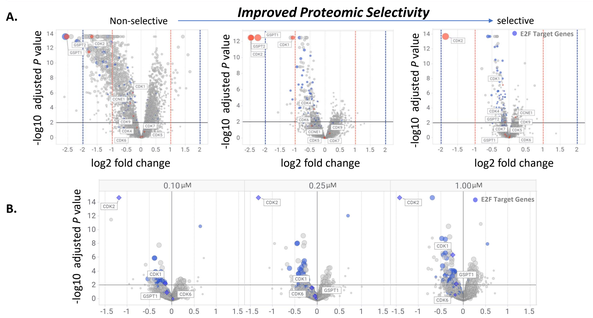
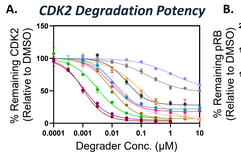


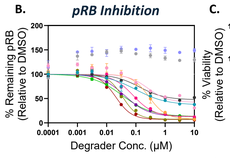
Figure 4. Selectivity profiling of CDK2 molecular glue degraders. (A) Quantitative proteomic profile in HCC1569 cell line treated for 24h with 1 μM of CDK2 degraders. **(B)** Treatment with the selective CDK2 molecular glue (PLX-513) results in dose dependent CDK2 degradation and inhibition of E2F target genes in the HCC1569 cell line. Cells were treated for 24h with varying concentrations of CDK2 degrader and analyzed by proteomics. Volcano plot represents the relationship between the $\log_2$ fold-change and the -$\log_{10}$ (*P* value*)*. E2F target genes are shown as blue dots.

CDK2 degradation correlates with pRB inhibition and anti-proliferation in CCNE1^{amp} cells

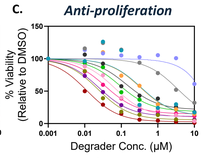
CDK2 Degradation Potency



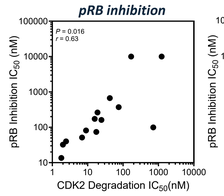
pRB Inhibition



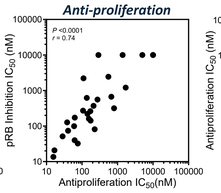
Anti-proliferation



CDK2 Degradation vs. pRB inhibition



pRB Inhibition vs. Anti-proliferation



CDK2 Degradation vs. Anti-proliferation

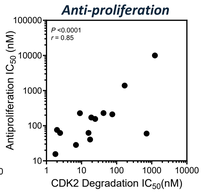
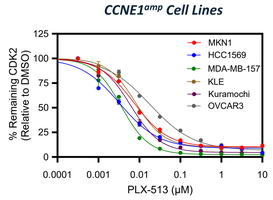


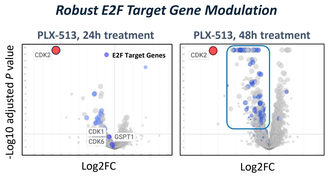
Figure 5. CDK2 degradation potency correlates with pRB inhibition and anti-proliferation in CCNE1^{amp} cell line. MKN1 cells were treated with different concentrations of CDK2 degraders and AlphaLISA assays were used to measure **(A)** CDK2 protein levels at 24h and **(B)** phospho-RB (S807/811) levels at 48h. **(C)** The cells were treated with varying concentrations of CDK2 degraders for 12 days and the cell viability was assessed using a clonogenic assay and crystal violet staining. **(D)** Correlation of CDK2 degradation DC_{50} and pRB inhibition IC_{50} and/or antiproliferative activity IC_{50} is shown in the graphs.

Optimized molecular glue shows potent CDK2 degradation across multiple CCNE1^{amp} cell lines

Potently Degrades CDK2 in CCNE1^{amp} Cell Lines



Selectively Degrades CDK2 and Induces Robust E2F Target Gene Modulation

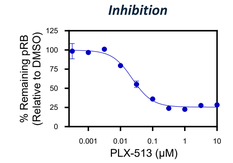


Cell Lines	CDK2 Degradation	
	DC_{50} (nM)	D_{max} (%)
MKN1 (Gastric)	8	89
HCC1569 (Breast)	3	94
MDA-MB-157 (Breast)	4	96
KLE (Endometrial)	9	90
Kuramochi (Ovarian)	6	95
OVCAR3 (Ovarian)	18	95

Figure 6. Potent degrader PLX-513 demonstrates potent and selective CDK2 degradation across multiple CCNE1^{amp} cell lines (A). Cells were treated with varying concentrations of PLX-513 for 24h and the CDK2 protein level was measured by AlphaLISA assay. DC_{50} and max degradation (D_{max}) are indicated in the table. **(B)** Quantitative proteomic profile in HCC1569 cell line treated for 24h or 48h with 1 μM of PLX-513. Volcano plot represents the relationship between the $\log_2$ fold-change and the -$\log_{10}$ (*P* value*)*. E2F target genes are indicated in blue dots.

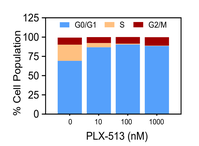
Potent CDK2 degradation leads to robust biomarker modulation in CCNE1^{amp} cell lines

A. RB Phosphorylation Inhibition

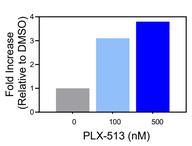


Compound ID	RB Phosphorylation IC_{50} (nM)
PLX-513	23

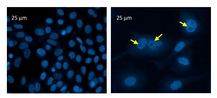
B. Cell Cycle Arrest



C. Cellular Senescence



D. Nuclear Enlargement

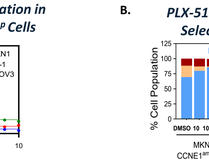


DMSO 100 nM PLX-513

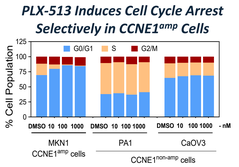
Figure 7. Potent CDK2 degradation inhibits RB phosphorylation, arrests cell cycle and induces cellular senescence in CCNE1^{amp} cell lines (A) MKN1 cells were treated with varying concentrations of PLX-513 for 48h and pRB levels were measured by AlphaLISA. **(B)** CDK2 degradation induces G1-arrest. Cell-cycle analysis was evaluated in the MKN1 cell line by flow cytometry, following 48h treatment with increasing concentrations of PLX-513. **(C)** PLX-513 treatment of the MKN1 cell line for 7 days results in increased senescence-associated β-galactosidase activity as measured by flow cytometry. **(D)** MKN1 cells were treated with 100 nM of PLX-513 for 12 days and morphology changes were analyzed by DAPI staining.

CDK2 molecular glue exhibits superior selectivity vs. CDK2 inhibitor in CCNE1^{amp} cell lines

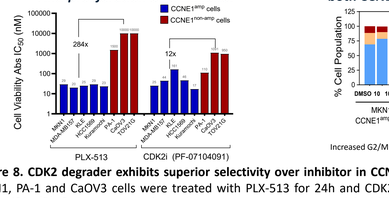
A. Comparable CDK2 Degradation in CCNE1^{amp} vs CCNE1$^{non\text{-}amp}$ Cells



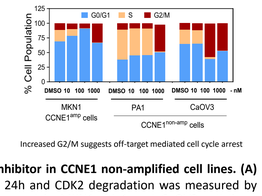
B. PLX-513 Induces Cell Cycle Arrest Selectively in CCNE1^{amp} Cells



C. PLX-513 Shows Selective Anti-proliferation Over Inhibitor



CDK2i Induces Cell Cycle Arrest in both CCNE1^{amp} and CCNE1$^{non\text{-}amp}$ Cells



Increased G2/M suggests off-target mediated cell cycle arrest

Figure 8. CDK2 degrader exhibits superior selectivity over inhibitor in CCNE1 non-amplified cell lines. (A) MKN1, PA-1 and CaOV3 cells were treated with PLX-513 for 24h and CDK2 degradation was measured by AlphaLISA. **(B)** Cell-cycle analysis by flow cytometry was assayed in MKN1, PA-1 and CaOV3 cell lines, following 48h treatment with varying concentrations of PLX-513 or CDK2 inhibitor **(C)** Antiproliferative activities of PLX-513 and CDK2 inhibitor were assessed in CCNE1^{amp} and CCNE1$^{non\text{-}amp}$ cell lines. IC_{50}*s* are shown as a bar graph for each cell line.

Molecular glue degrader demonstrates potent CDK2 degradation *in vivo*

Deep and Sustained CDK2 Degradation

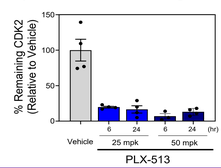


Figure 9. *In vivo* degradation of CDK2 in CDX mouse model. Mice implanted with HCC1599 xenograft model were dosed with 25 or 50 mg/kg PLX-513 QDx2 via oral administration. Tumor samples were collected 6h or 24h after last dose and CDK2 levels were assessed using the AlphaLISA assay. (n=4 in each group)

Conclusions

- Novel CDK2 molecular glues were identified that selectively degrade CDK2 resulting in preferential anti-proliferative activity in CCNE1 amplified cells with superior activity relative to ATP-competitive small molecule CDK2 inhibitors
- CDK2 molecular glue degraders induce biomarker modulation including inhibition of RB phosphorylation and cell cycle arrest in CCNE1^{amp} cell lines that is dependent on potency and depth of CDK2 degradation
- Oral administration of CDK2 degraders result in potent and dose dependent degradation *in vivo*
- Data supports the potential for CDK2 molecular glue degraders to treat CDK4/6 inhibitor-naïve and resistant HR+/HER2- breast cancer, and CCNE1 amplified patient populations

Plexium