Ultra-High-Throughput Miniaturized Cell-Based Assays to Discover CDK2 Molecular Glue Degraders

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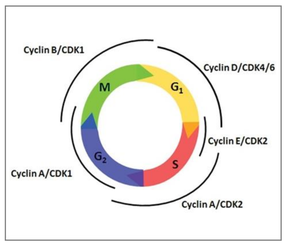
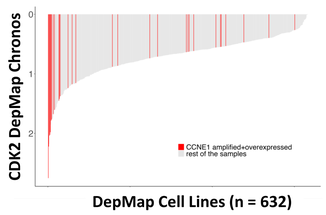
Introduction

- CDK2 associates with cyclin E (CCNE1) to regulate cell cycle progression. Aberrant Cyclin E levels activate CDK2 and correlate with poor survival (subset of Ovarian and Breast)
- Tumor cell lines with CCNE1 amplifications are sensitive to loss of CDK2
- CDK2 degrader has 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. Patients on CDK4/6 inhibitors for ER+HER- breast cancer develop resistance which can be driven by cyclin E overexpression

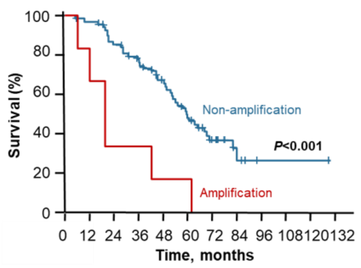
CDK2 Regulates G1/S Cell Cycle Transition



Tumor Cell Lines with CCNE1^{amp} Sensitive to CDK2



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



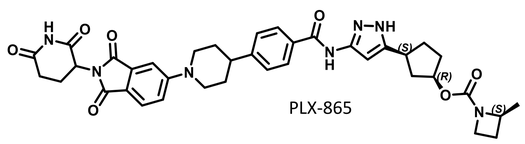
Tumor Type	CCNE1^{amp} Frequency*
Uterine	40%
Ovarian	19%
Gastric	11%
Esophageal	8%
Endometrial	8%
Sarcoma	8%
Bladder	6%
NSCLC	5%

*cBioPortal frequency of CCNE1 amplification

Benefits of a CDK2 degrader:

- Increase selectivity
- Improve efficacy from reducing CDK2 levels to drive deeper pRb suppression
- Address potential future resistance mechanisms

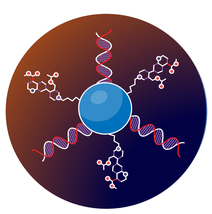
Identification of Selective CRBN-based CDK2 PROTAC

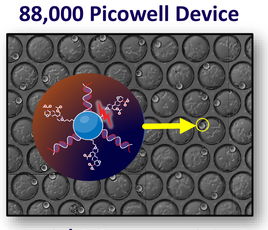


PLX-865

- Potent CDK2 degrader
- *in vivo* CDK2 modulation in tumor model (IP dosing)
- **Suboptimal selectivity (CDK1 inhibition/degradation)**
- **Highly lipophilic (LipE < 3) with limited oral exposure**

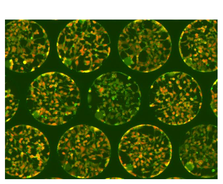
Picowell Platform





88,000 Picowell Device

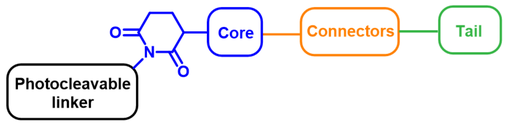
1 Bead / 1 Compound Per Well



Protein of Interest Depletion

Decoding – Compound identification

Cereblon-based Compound Library



Library distribution

Property	Mean
logD	2.5
logP	2.8
MW	486.7
HBD	1.4
HBA	7.8
TPSA	94.1
Rotatable Bonds	8.7
Fraction sp3	0.36

Cereblon binder / Core

- Literature and proprietary scaffolds

Connector

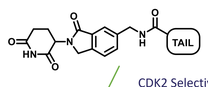
- Allow for flexible chemistry and installation of broad diversity in the tail region

Tail

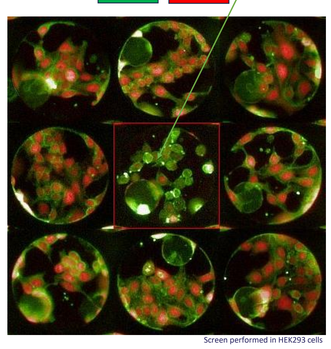
- Interacting group that reaches out to interact with the protein of interest
- Consists of broad diversity, or specific groups based on targets of interest to Plexium

Our CRBN bead library has reached one million compounds

Hit Identification – PLX-519



CDK2 Selective Degrader

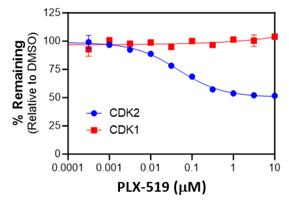


Screen performed in HEK293 cells

High throughput Imaging and Data Analysis

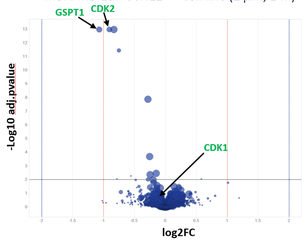
Selective Degradation

HiBiT cell line (24 hrs)

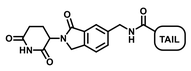


CDK2 Degradation

MDA-MB-157 CCNE1^{amp} cell line (1 µM, 24h)



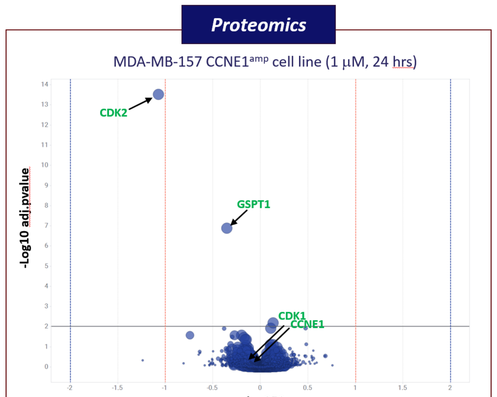
Hit Optimization – PLX-322



	PLX-519	*Tail SAR* PLX-581	*Novel Scaffold* PLX-322
CDK2 HiBit DC_{50}, D_{max}, CDP	**79 nM, 46%, 4.1**	**18 nM, 68%, 6.6**	**61 nM, 61%, 5.5**
CDK1 / GSPT1	GSPT1 degradation	>10 µM	>10 µM
CRBN HTRF EC50	1.6 µM	0.84 µM	0.58 µM
HLM CL (mL/min/kg)	< 8.6	13.1	<8.6
Kin Solubility 7.4	44 µM	<2 µM	64 µM
Mouse PK	NA	NA	Good exposure

Proteomics

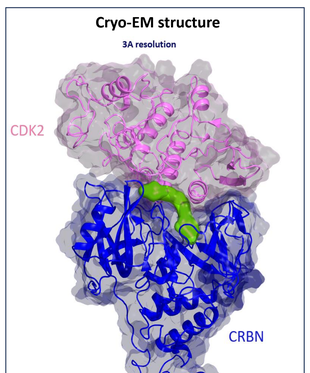
MDA-MB-157 CCNE1^{amp} cell line (1 µM, 24 hrs)



- ***Good selectivity over GSPT1***
- ***Negligible CCNE1 and CDK1 degradation***

Cryo-EM structure

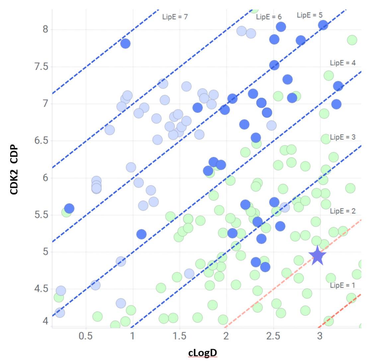
3A resolution



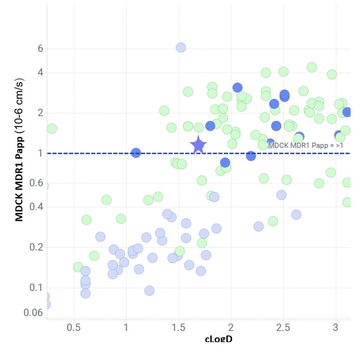
Suboptimal potency observed for 62322 in CCNE1^{amp} cell lines

Identification of a more drug-like series

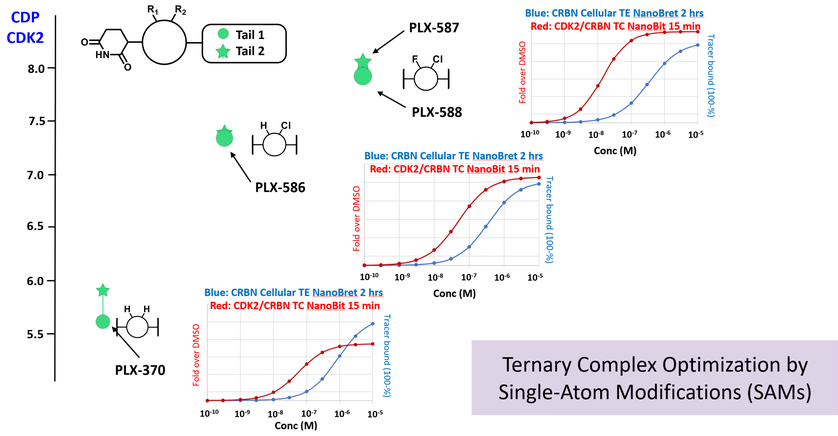
CDK2 LipE Plot



Permeability

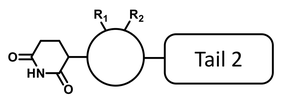


Cell Ternary Complex Formation



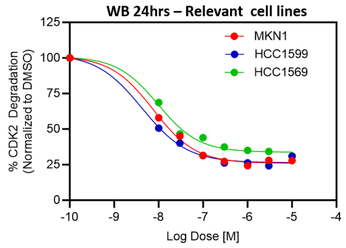
Ternary Complex Optimization by Single-Atom Modifications (SAMs)

Identification of PLX-587



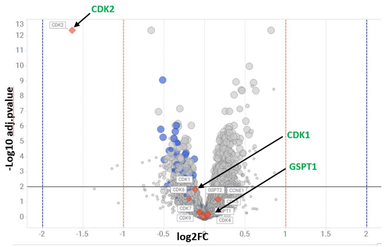
MW	<550
CRBN HTRF	0.361 µM
CDK2 HiBit 24h DC_{50}, D_{max} , CDP	■ 0.008 µM, 80%, 8.0
GSPT1 HiBit 24h DC_{50}, D_{max}	■ >10 µM, 26%
In vitro ADME	Good
Mouse PO 10 mpk	Good

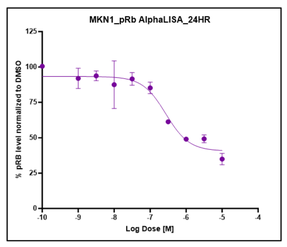
WB 24hrs – Relevant cell lines



CDK2 24h DC_{50}, D_{max} = 5-10 nM, 70-75%

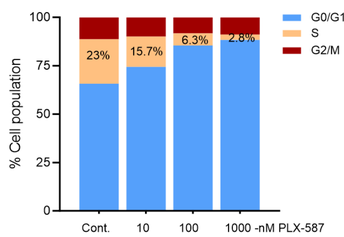
1 µM, 24 hrs, HCC1569 cells





pRb 24h IC_{50}, I_{max} = 271.5 nM, 65%

MKN1 _48HRS treatment



PLX-587 is currently being evaluated in different *in vivo* models

Conclusions

- Ultra high-throughput miniaturized picowell screen provided multiple starting points for developing a CDK2 CRBN molecular glue.
- Established a medicinal chemistry path leading to PLX-587 that demonstrates an efficient and orally bioavailable selective CDK2 degrader.
- TGI studies CCNE1^{amp} breast cancer xenograft are ongoing (single agent/combo).