Discovery of novel small molecules that recruit DCAF11 for selective degradation of BRD4

Gregory Parker, Julia Toth, Geoffray Leriche, Sarah Fish, Aleksandar Jamborcic, Gabrielle Blanco, Taylor Kampert, Elizabeth Daniele, Kenneth Steadman, Xiaoming Li, Linette Yang, Stephen Chien, Alejandro Dearie, Kenneth Chng, Erika Green, Michael Hocker, Yi Zhang, Peggy Thompson, Simon Bailey

#225

Plexium, San Diego, CA

gparker@plexium.com

Abstract

Background: Targeted protein degradation (TPD) using the endogenous ubiquitin (Ub) proteasome system (UPS) is a rapidly growing drug discovery approach to eliminate pathogenic proteins. Strategies for TPD have focused on designing heterobifunctional PROTACs that utilize ligand binding to select E3 ligases (e.g., cereblon and VHL), often resulting in compounds with poor drug-like properties. Monovalent degraders represent an alternative approach, in which small molecules are designed to bind the target protein and induce its degradation through the recruitment of an E3 ligase complex. Monovalent degraders generally have better physico-chemical properties than PROTACs; however, designing compounds to induce degradation is not well precedented and relies on serendipitous discovery.

Methods: An E3 ligase agnostic chemical library was screened using our ultra-high throughput cell-based screening (uHTS) platform. Hits were identified by measuring protein degradation of BRD4 using immunofluorescence. A Ub pathway focused CRISPR screen was used to identify the E3 ligase responsible for compound-induced degradation of BRD4. The degradation mechanism was validated using western blot, luciferase-based protein-protein interaction (PPI) and mutational analyses. *In vitro* antitumor activity was assessed by proliferation and apoptosis assays. NOD/SCID mice were implanted sub-Q with acute myeloid leukemia (AML) xenograft model MV-4-11 and treated with compounds administered QD.

Results: uHTS resulted in identification of selective monovalent degraders that target the bromodomain extra-terminal (BET) protein, BRD4. Degrader series optimization produced PLX-3618, which demonstrated potent degradation of BRD4, without depleting BET family members BRD2 and BRD3. Proteasome and neddylation inhibitors confirmed degradation was mediated via the UPS, and a Ub-focused CRISPR screen identified $CUL4^{DCAF11}$ as the E3 complex responsible for PLX-3618 induced degradation of BRD4. PPI studies verified a BRD4/PLX-3618/DCAF11 ternary complex; BRD4 point mutational analyses provided further insights into the DCAF11-mediated degradation mechanism. Degradation of BRD4 by PLX-3618 resulted in downregulation of the MYC oncogene and potent anti-proliferative activity against a panel of tumor cell lines, with high sensitivity observed in AML. *In vivo*, PLX-3618 resulted in complete tumor regression, whereas a pan-BET inhibitor only resulted in tumor growth inhibition without regression. PK/PD analyses provided insight into the unique exposure/response profile of targeted protein degraders.

Conclusion: These results demonstrate the efficient discovery of novel monovalent degraders using our proprietary platform. Characterization of the degradation mechanism highlights the discovery of DCAF11 as a novel E3 ligase substrate receptor amenable to recruitment and degradation of BRD4.

Plexium's screening platform and initial hit identification, validation, and optimization

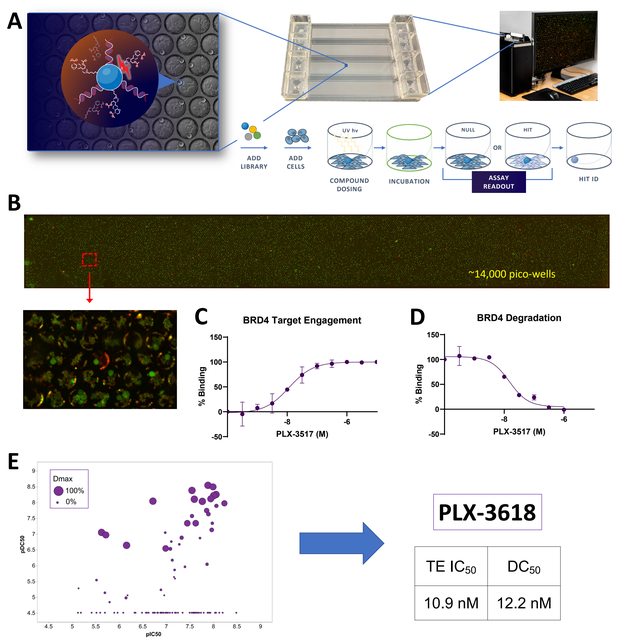


Figure 1: A. Overview of the Plexium ultra-high throughput screening workflow. **B.** Lane view of Plexium Picowell device with increased magnification showing example of a hit well (BRD4 = green; Tubulin = red) **C.** NanoLuc-BRD4 target engagement binding curve of screening hit compound, PLX-3517. Bars represent standard deviation (SD) **D.** BRD4 degradation using PLX-3517 in HiBiT-BRD4-HEK-293 cells. Bars represent SD. **E.** Optimization of degrader series. Scatter plot of BRD4 target engagement IC_{50} vs. BRD4 degradation DC_{50}. Dot size represents the magnitude of degradation (Dmax). Optimization yielded lead tool compound, PLX-3618.

Plexium monovalent degraders require the proteasome to selectively degrade BRD4

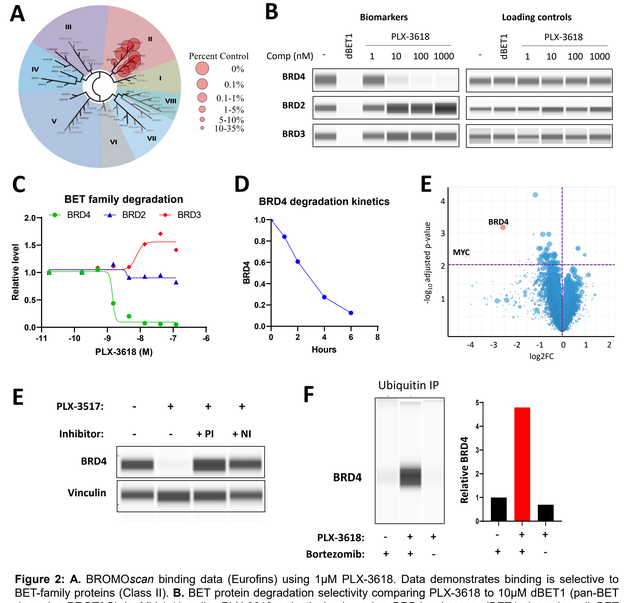


Figure 2: A. BROMO*scan* binding data (Eurofins) using 1μM PLX-3618. Data demonstrates binding is selective to BET-family proteins (Class II). **B.** BET protein degradation selectivity comparing PLX-3618 to 10μM dBET1 (pan-BET degrader PROTAC) in MV-4-11 cells. PLX-3618 selectively degrades BRD4, whereas dBET1 degrades all BET proteins. Loading controls were vinculin (BRD4) and GAPDH (BRD2 and BRD3). **C.** Degradation dose response curves, comparing BRD2, BRD3, and BRD4 in MV-4-11 cells treated with PLX-3618 for 24h. Data quantified from western blot analyses. **D.** BRD4 degradation rate in MV-4-11 cells treated with 15nM PLX-3618. **E.** Proteomic changes in MV-4-11 in response to 7h exposure of 100nM PLX-3618. **E.** Degradation is mediated by the proteasome. HEK-293T cells were incubated +/- proteasome inhibitor (PI; 100nM bortezomib) or neddylation inhibitor (NI; 1μM MLN4924) for 2h, followed by a 6h incubation with 100nM PLX-3517. **F.** PLX-3618 induces BRD4 ubiquitination. Ubiquitinated proteins were immunoprecipitated following PLX-3618 treatment in HEK-293T cells, and subsequently subjected to western blot analysis, probing for BRD4.

PLX-3618 recruits DCAF11 for degradation of BRD4

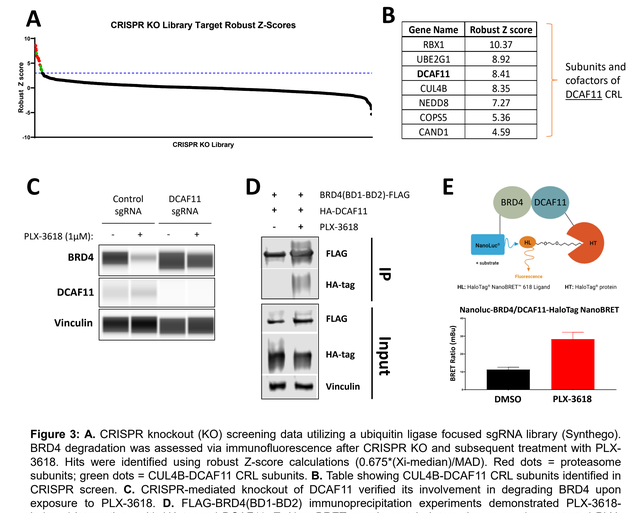


Figure 3: A. CRISPR knockout (KO) screening data utilizing a ubiquitin ligase focused sgRNA library (Synthego). BRD4 degradation was assessed via immunofluorescence after CRISPR KO and subsequent treatment with PLX-3618. Hits were identified using robust Z-score calculations (0.675*(Xi-median)/MAD). Red dots = proteasome subunits; green dots = CUL4B-DCAF11 CRL subunits. **B.** Table showing CUL4B-DCAF11 CRL subunits identified in CRISPR screen. **C.** CRISPR-mediated knockout of DCAF11 verified its involvement in degrading BRD4 upon exposure to PLX-3618. **D.** FLAG-BRD4(BD1-BD2) immunoprecipitation experiments demonstrated PLX-3618-induced interactions with HA-tagged DCAF11. **E.** NanoBRET protein-protein interaction assay demonstrated PLX-3618-induced interactions between NanoLuc-BRD4 and HaloTag-DCAF11.

PLX-3618-mediated degradation of BRD4 requires binding to both bromodomains

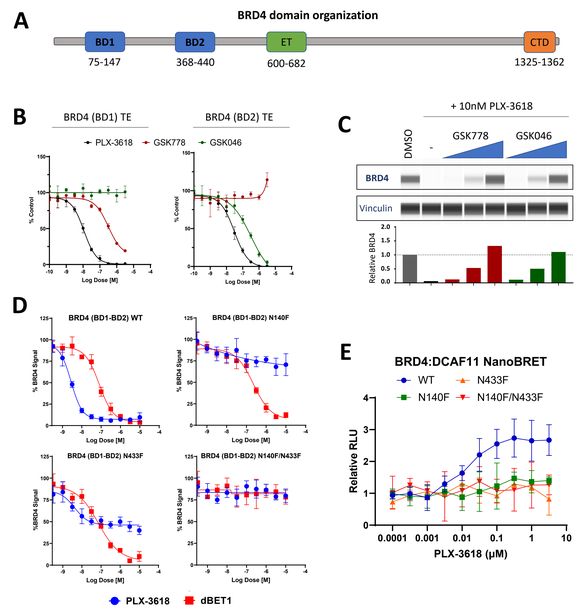


Figure 4: A. Domain organization of BRD4 with corresponding amino acid ranges. BRD4 contains two N-terminal bromodomains (BD1 and BD2), an extra-terminal domain (ET), and C-terminal domain (CTD). **B.** BRD4 bromodomain (BD) binding selectivity. PLX-3618 binds both BD1 and BD2 in NanoBRET target engagement assays (Promega); tool inhibitor GSK778 is BD1-selective; tool inhibitor GSK046 is BD2-selective. **C.** Competition experiment using BD-selective tool inhibitors. Both BD1 and BD2-selective inhibitors compete for PLX-3618-mediated degradation of BRD4. **D.** Bromodomain-specific point mutations, known to abrogate binding, were introduced into Nanoluc protein constructs and used to monitor PLX-3618-mediated degradation. Mutational analysis confirmed the requirement of both bromodomains for full degradation of BRD4 by PLX-3618, whereas the PROTAC, dBET1, requires only one. **E.** PPI NanoBRET assay, with constructs harboring bromodomain point mutations, show that binding to both bromodomains are required for efficient BRD4:DCAF11 ternary complex formation.

Potent anti-tumor activity identified in AML

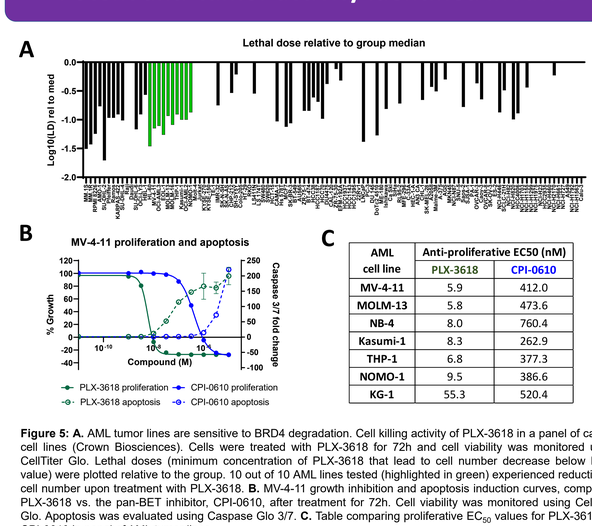


Figure 5: A. AML tumor lines are sensitive to BRD4 degradation. Cell killing activity of PLX-3618 in a panel of cancer cell lines (Crown Biosciences). Cells were treated with PLX-3618 for 72h and cell viability was monitored using CellTiter Glo. Lethal doses (minimum concentration of PLX-3618 that lead to cell number decrease below Day0 value) were plotted relative to the group. 10 out of 10 AML lines tested (highlighted in green) experienced reduction in cell number upon treatment with PLX-3618. **B.** MV-4-11 growth inhibition and apoptosis induction curves, comparing PLX-3618 vs. the pan-BET inhibitor, CPI-0610, after treatment for 72h. Cell viability was monitored using CellTiter Glo. Apoptosis was evaluated using Caspase Glo 3/7. **C.** Table comparing proliferative EC_{50} values for PLX-3618 vs. CPI-0610 in panel of AML tumor lines

PLX-3618 elicits potent anti-tumor activity *in vivo*

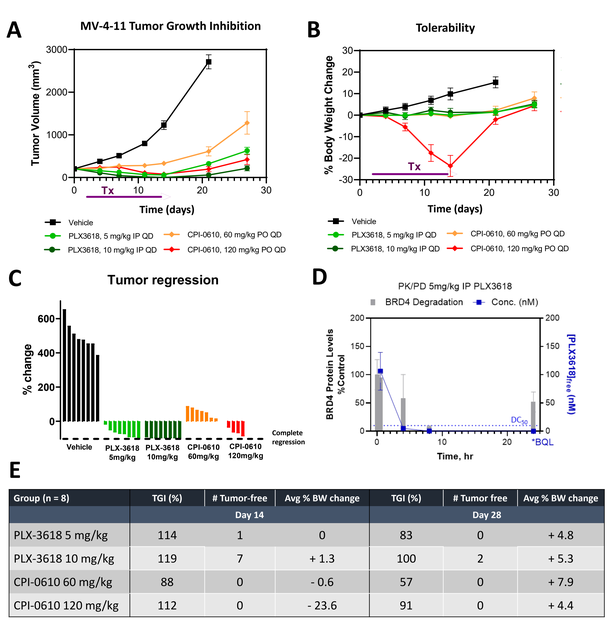
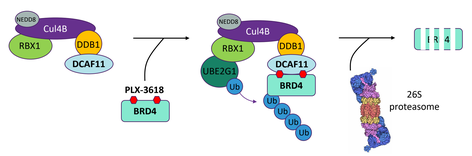


Figure 6: A. PLX-3618 potently inhibits tumor growth. NOD/SCID mice bearing MV-4-11 subcutaneous tumors were treated with PLX-3618 or CPI-0610 for 14 days. Treatment was terminated, and tumor regrowth was monitored to Day 28. **B.** Average body weight changes for the different treatment groups. Both doses of PLX-3618 were well tolerated; high dose of CPI-0610 was not tolerated. **C.** Individual % change tumor volume waterfall plot illustrates tumor regression with PLX-3618 treated animals after 14 days of treatment. Tolerated doses of CPI-0610 only led to tumor inhibition, without regression. **D.** PK/PD plot with 5 mg/kg IP dose of PLX-3618. Data demonstrates rapid and sustained depletion of BRD4. **E.** Table of *in vivo* study values (TGI = tumor growth inhibition; # Tumor-free = number of animals with no measurable tumor; BW = body weight).

Summary



- Plexium's ultra high-throughput screening platform was used to identify potent and selective BRD4 monovalent degraders
- BRD4 degradation led to potent anti-proliferative activity in AML tumor models, both *in vitro* and *in vivo;* complete tumor regression observed with well-tolerated doses
- A cullin-RING ligase complex containing the E3 substrate receptor, DCAF11, was identified as the required CRL for PLX-3618-mediated degradation of BRD4
- Mechanistic studies demonstrate BRD4:PLX-3618:DCAF11 ternary complexes, as well as unique bromodomain requirements for efficient target degradation
- Insights obtained from the BRD4 monovalent degrader discovery program are being applied to enable Plexium's drug discovery pipeline

Plexium