PLX-3618, a potent, selective monovalent BRD4 degrader demonstrates activity in models of prostate cancer

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Abstract

Prostate cancer is the second most common cause of cancer related deaths in men in the United States. Pathogenesis is driven by the androgen receptor (AR), which has led to front-line treatment modalities that are based on androgen deprivation therapy (ADT). About 10-20% of all prostate cancers evolve to resist ADT and are classified as castration-resistant prostate cancer (CRPC) indicating the continued need for new treatment options. Bromodomain-containing protein 4 (BRD4) is an acetylated-chromatin associating protein that is involved in transcriptional elongation, mRNA splicing, epigenetic bookmarking, and super-enhancer activity. The BRD4 protein has been shown to both bind and colocalize with AR at androgen response elements (AREs) on chromatin. Furthermore, elevated BRD4 expression is prognostic of increased prostate specific antigen (PSA) levels following radical prostatectomy and is correlated with higher Gleason scores and poor overall survival. The intertwined activity of BRD4 with multiple essential driver mechanisms of prostate cancer suggests it may be a key target for developing novel therapeutics. Using our ultra-high throughput cell-based screening platform, which directly measures degradation of pathogenic proteins upon exposure to diverse chemical libraries, we identified a series of novel monovalent BRD4 degraders that was optimized to produce PLX-3618. A cancer cell panel screen for antiproliferative effects of PLX-3618 indicated enhanced sensitivity in subsets of prostate cancer lines. PLX-3618 elicited selective, rapid, and deep degradation of BRD4 protein in prostate cancer cell models, without degrading the closely related BRD2 and BRD3 proteins. Addition of either proteosome or neddylation inhibitors blocked BRD4 degradation indicating a ubiquitin-proteosome system mediated clearance mechanism. Degradation of BRD4 led to sustained multimodal inhibition of the AR pathway and disruption of key oncogene enhancer networks. When compared to pan-BET inhibitors, treatment of select prostate cancer cell lines with PLX-3618 resulted in increased levels of the tumor suppressors p53 and p21, an aberrant DNA damage response, and substantially amplified apoptosis. Finally, in in vivo preclinical models of prostate cancer, PLX-3618 showed far superior efficacy over a pan-BET inhibitor. Taken together, the selective degradation of BRD4 via the potent monovalent degrader PLX-3618 may represent a novel strategy in treating prostate cancer.

Plexium's screening platform enables novel degrader discovery and hit optimization

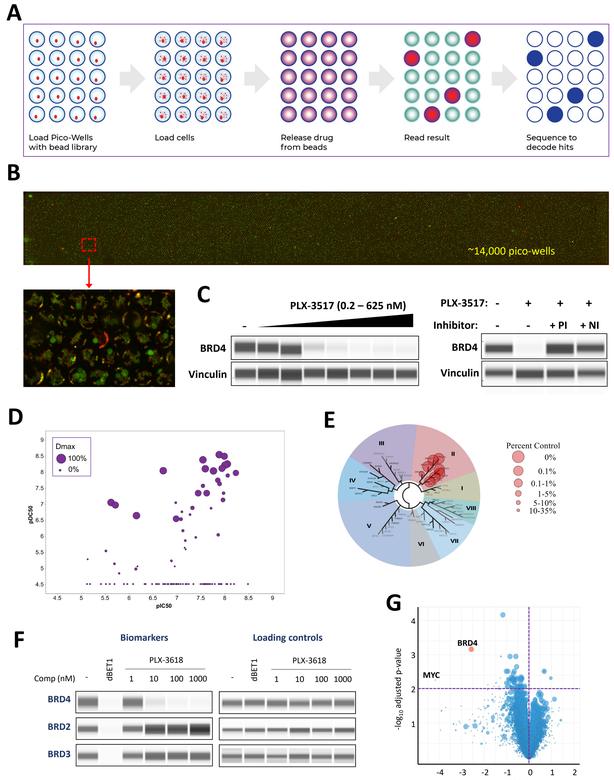
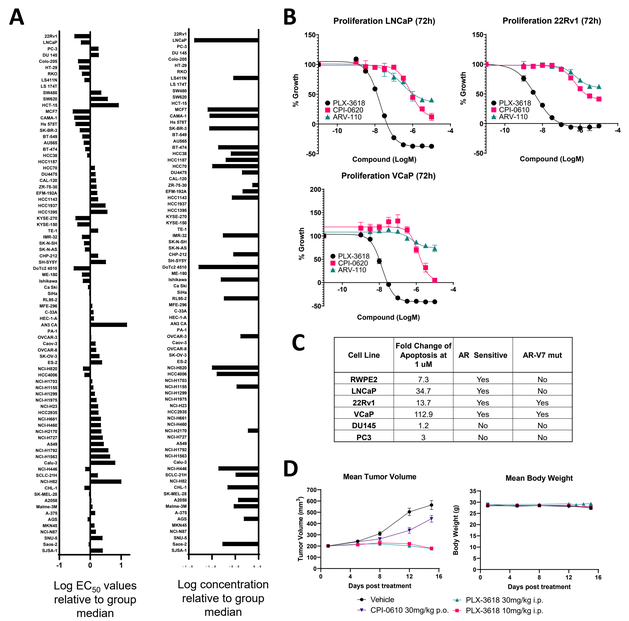


Figure 1: A. Overview of the Plexium ultra-high throughput screening workflow. **B.** Pico-well lane view with increased magnification showing example of a hit well (BRD4 = green; Tubulin = red) **C. Left Panel** BRD4 protein levels with increasing concentrations of PLX-3517 at 24h in HEK-293T cells. Protein was detected using a Jess Simple Western (ProteinSimple), with vinculin as a loading control. **C. Right Panel** 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. **D.** 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). **E.** BROMO*scan* binding data (Eurofins) using 1μM of the optimized compound, PLX-3618. **F.** BET protein degradation selectivity comparing PLX-3618 to 10μM dBET1 (pan-BET degrader PROTAC) in LNCaP cells. Vinculin was used as a loading control for BRD4; GAPDH was used as a loading control for BRD2 and BRD3 **G.** Proteomic changes in LNCaP in response to 6 hr exposure to 100nM PLX-3618.

Prostate cancer cells are sensitive to PLX-3618-mediated BRD4 degradation



Cell Line	Fold Change of Apoptosis at 1 uM	AR Sensitive	AR-V7 mut
RWPE2	7.3	Yes	No
LNCaP	34.7	Yes	No
22Rv1	13.7	Yes	Yes
VCaP	112.9	Yes	Yes
DU145	1.2	No	No
PC3	3	No	No

Figure 2: A Left Panel. Anti-proliferative activity of PLX-3618 in a panel of cancer cell lines (Crown OmniScreen). Cells were treated with PLX-3618 for 72h and cell viability was monitored using CellTiter Glo (Promega). Log(EC_{50}) values plotted relative to the group median (0 = 64.7nM). **A Right Panel.** Minimum concentration of PLX-3618 that lead to cell number decrease below Day0 value. Log concentration plotted relative to the group median (0 = 1μM). **B.** Prostate cancer cells were treated with PLX-3618, CPI-0610 (pan-BET inhibitor) and ARV-110 (an Androgen Receptor PROTAC) for 72h and cell viability was measured using CellTiter Glo. **C.** Fold increase of apoptosis values in prostate cell lines in response to 72h exposure to PLX-3618 at 1 uM corresponds to androgen sensitivity. **D.** PLX-3618 elicits potent tumor growth inhibition in LNCaP xenograft model. Tumor-bearing mice were dosed daily for 14 consecutive days with either 10 or 30 mg/kg PLX-3618 and compared to daily dosing of 30 mg/kg CPI-0610. Right panel demonstrates that all treatment groups were well tolerated as evidenced by no significant body weight changes.

Role of BRD4 in prostate cancer super enhancer networks

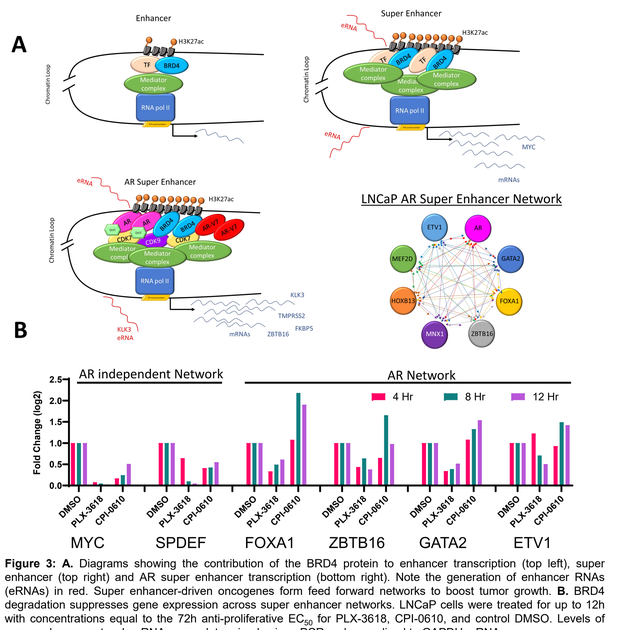


Figure 3: A. Diagrams showing the contribution of the BRD4 protein to enhancer transcription (top left), super enhancer (top right) and AR super enhancer transcription (bottom right). Note the generation of enhancer RNAs (eRNAs) in red. Super enhancer-driven oncogenes form feed forward networks to boost tumor growth. **B.** BRD4 degradation suppresses gene expression across super enhancer networks. LNCaP cells were treated for up to 12h with concentrations equal to the 72h anti-proliferative EC_{50} for PLX-3618, CPI-0610, and control DMSO. Levels of super enhancer network mRNAs were determined using qPCR and normalized to GAPDH mRNA.

PLX-3618 represses prostate super enhancers

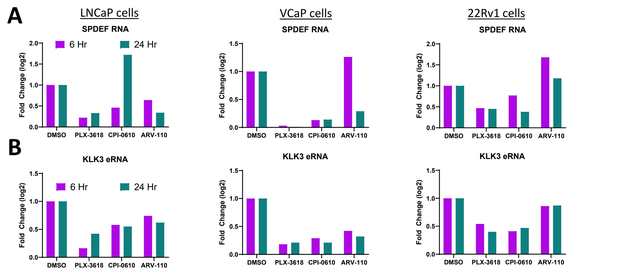
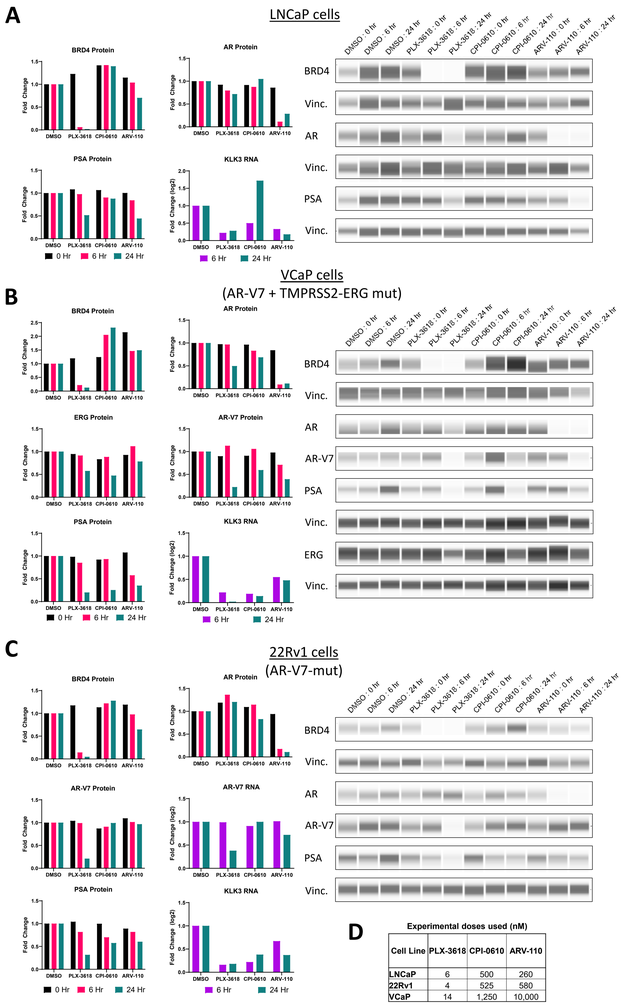


Figure 4: A. Prostate super enhancer network gene SPDEF is highly-repressed by BRD4 modulation. Prostate cancer cells were treated for up to 24h with concentrations equal to the 72h anti-proliferative EC_{50} for each cell line. Effects of PLX-3618, CPI-0610 and ARV-110 treatment on SPDEF mRNA levels determined by qPCR in LNCaP, VCaP and 22Rv1 cells (left, middle and right panels). **B.** The KLK3 Enhancer RNA created by super enhancer activity on upstream of the promoter for KLK3 showing directly loss of super enhancer activity. The eRNA level was determined by qPCR in LNCaP, VCaP and 22Rv1 cells (left, middle and right panels).

PLX-3618 potently inhibits androgen signaling



Cell Line	PLX-3618	CPI-0610	ARV-110
LNCaP	6	500	260
22Rv1	4	525	580
VCaP	14	1,250	10,000

Figure 5: Suppression of BRD4 blocks AR signaling. Prostate cancer cells were treated up to 24h with concentrations equal to the 72h anti-proliferative EC_{50} for each cell line. The VCaP and 22Rv1 cell lines both express Androgen Receptor Variant 7 (AR-V7) which is missing the AR ligand binding domain and is constitutively active without hormone binding. Effects of PLX-3618, CPI-0610 and ARV-110 treatment on BRD4 and the androgen pathway normalized to Vinculin protein levels seen in **A** LNCaP, **B** VCaP, **C** 22Rv1 shown by representative western blot (right pictographs), protein quantification and qPCR (left panels). **D.** Table shows doses of PLX-3618, CPI-0610 and ARV-110 used in the above experiments.

MYC activity is strongly suppressed by PLX-3618

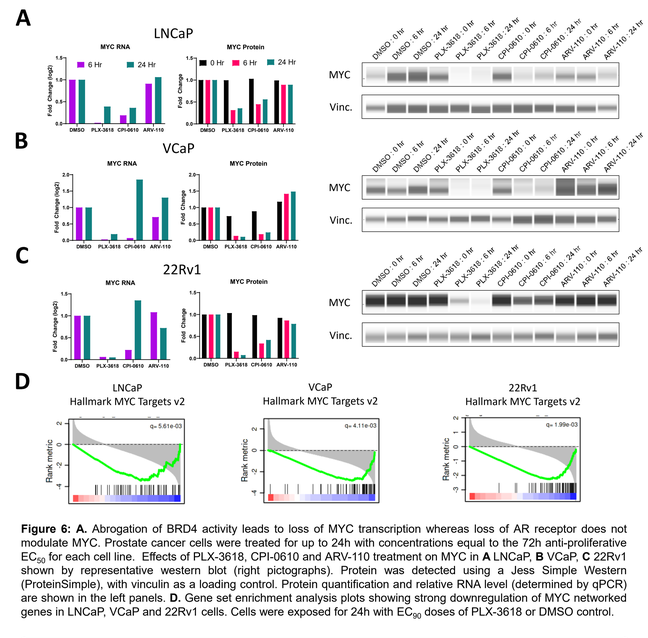


Figure 6: A. Abrogation of BRD4 activity leads to loss of MYC transcription whereas loss of AR receptor does not modulate MYC. Prostate cancer cells were treated for up to 24h with concentrations equal to the 72h anti-proliferative EC_{50} for each cell line. Effects of PLX-3618, CPI-0610 and ARV-110 treatment on MYC in **A** LNCaP, **B** VCaP, **C** 22Rv1 shown by representative western blot (right pictographs). Protein was detected using a Jess Simple Western (ProteinSimple), with vinculin as a loading control. Protein quantification and relative RNA level (determined by qPCR) are shown in the left panels. **D.** Gene set enrichment analysis plots showing strong downregulation of MYC networked genes in LNCaP, VCaP and 22Rv1 cells. Cells were exposed for 24h with EC_{90} doses of PLX-3618 or DMSO control.

PLX-3618 modifies DNA damage response

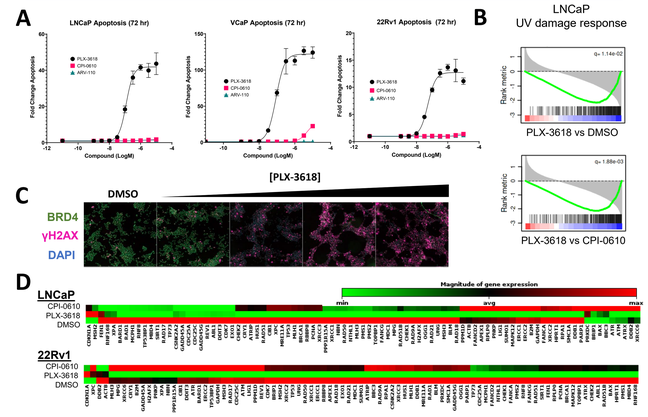


Figure 7: A. The mechanism of apoptosis in prostate cancer cells caused by BRD4 degradation is not driven by inhibition of MYC or AR as CPI-0610 and ARV-110 were unable to induce cytotoxic effects. Apoptosis was measured in prostate cancer cells that were treated for 72h with PLX-3618, CPI-0610 and ARV-110 using Caspase-Glo® Assay System (Promega). **B.** Gene set enrichment analysis plots showing strong downregulation of UV damage response genes in LNCaP cells when treated with PLX-3618 but not CPI-0610, cells were exposed for 24h with EC_{90} doses of PLX-3618, CPI-0160 or DMSO control. **C.** DNA damage was confirmed by γH2AX immunofluorescent staining, loss of BRD4 protein corresponds with increased DNA damage. **D. Top Panel** LNCaP cells were treated at the 72h antiproliferative EC_{70} doses for 12h, RNA was extracted, and qPCR performed with a DNA damage pathway array (Qiagen). **D. Bottom Panel.** 22Rv1 cells were treated with 72h antiproliferative EC_{90} doses for 24h, RNA was extracted, and qPCR performed with a DNA damage pathway array using 8 housekeeping genes for normalization (Qiagen). Heatmaps indicate near total loss of DNA damage response genes in response to BRD4 degradation but not BRD4 inhibition.

Summary

PLX-3618 is highly potent monovalent degrader of BRD4. In androgen sensitive prostate cancer cells, the rapid loss of BRD4 protein leads to abrogation of androgen signaling by inhibition of transcription and AR super enhancer activity. Additionally, PLX-3618 suppresses MYC transcription and MYC downstream network activity. However, MYC and AR loss only contribute to the cytostatic effects of PLX-3618 as CPI-0610 and ARV-110 did not induce strong apoptosis. The BRD4 degradation cytotoxicity is caused by the modulation of the DNA damage response network and subsequent increased DNA damage. The ability to suppress androgen and cancer super enhancers, while inducing DNA damage, suggests that degradation of BRD4 may represent a novel approach to treating prostate cancer.

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