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BRD4 Degradation by PROTACs Represents a More Effective Therapeutic Strategy than BRD4 Inhibitors in Ovarian Cancer

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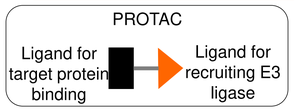
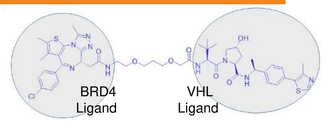
Abstract

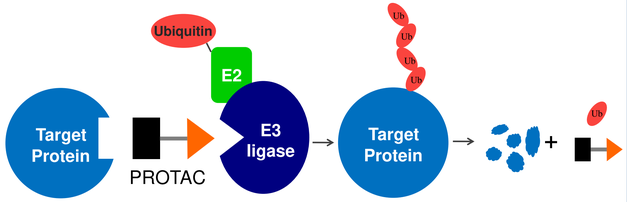
- BRD4, a member of the bromodomain and extraterminal domain (BET) family of proteins, has emerged as an attractive oncology target
- BET inhibitors have shown promising results in a number of preclinical settings, including ovarian cancer (OvCa)
- We have designed Proteolysis Targeting Chimera (PROTACs) against BRD4, which are heterobifunctional small molecule degraders containing a BRD4 binding moiety and a ligand for the E3 ubiquitin ligase VHL
- PROTAC treatment leads to rapid and efficient degradation of BRD4 across OvCa cell lines ($0.1nM<DC_{50}<1nM$)
- BRD4 PROTACs have more potent anti-proliferative activity than BET inhibitors in OvCa cell lines. However, this activity is highly variable ($0.6nM<EC_{50}<1\mu M$)
- We have performed RNA-sequencing on 5 OvCa cell lines to identify a genetic signature correlated with sensitivity to our BRD4 PROTACs
- BCLxL, recently shown to predict BET inhibitor sensitivity in cancer, emerges as a potential clinical biomarker candidate in our analysis
- Finally, BRD4 PROTACs are potent in vivo agents and efficacious in an A2780 tumor model of OvCa wherein BET inhibitors are inactive

PROTAC: PROteolysis TArgeting Chimera





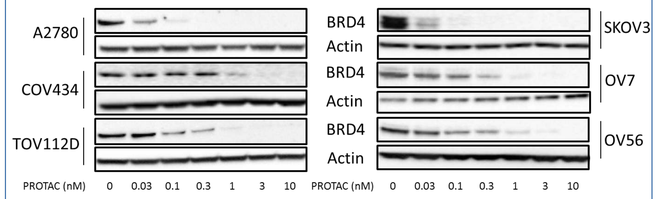
Representative BRD4 PROTAC



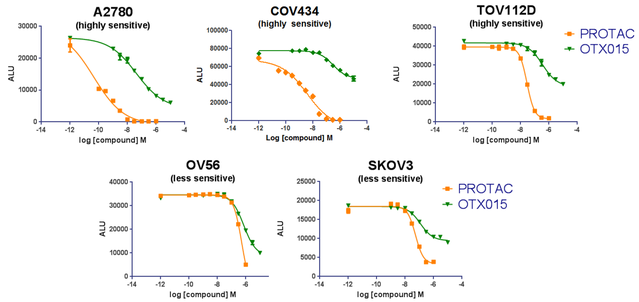
E3 Ligase is recruited to the target protein by PROTAC

Tagged target is recognized and degraded by proteasome

BRD4 PROTACs are potent degraders in OvCa cell lines



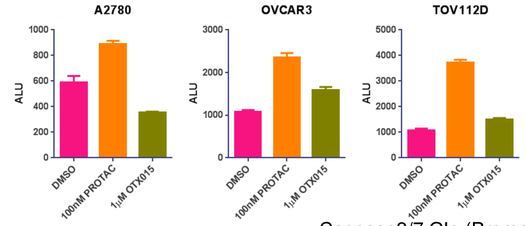
PROTAC mediated BRD4 degradation is highly anti-proliferative compared to BET inhibitor OTX015 in a subset of OvCa cell lines



OvCa Cell Line	PROTAC EC_{50} (nM)	OTX015 EC_{50} (nM)	Ratio (OTX/PROTAC)
A2780	0.6	155	258
COV434	1	304	304
Kuramochi	15	60	4
OV7	19	490	26
OVSAHO	29	270	9
TOV112D	30	600	20
OVCAR3	37	380	10
OAW28	43	438	10
SKOV3	75	118	1.6
OVKATE	120	237	2
COV318	375	780	2.1
OV56	528	721	1.4

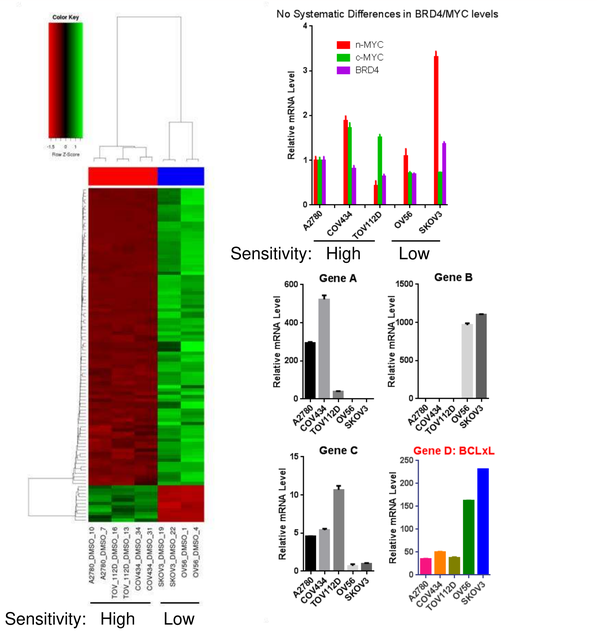
CellTiterGlo Assay (Promega)

PROTAC treatment results in pronounced apoptosis in sensitive OvCa cell lines

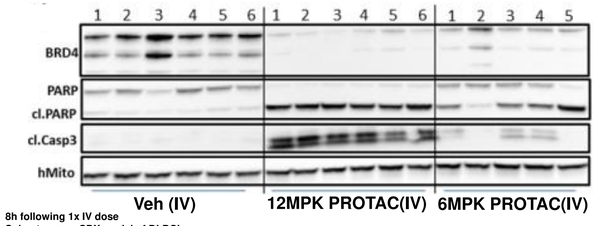


Caspase3/7 Glo (Promega)

Genes with known roles in OvCa tumorigenesis and progression are differentially expressed in highly PROTAC sensitive vs. less sensitive OvCa cell lines

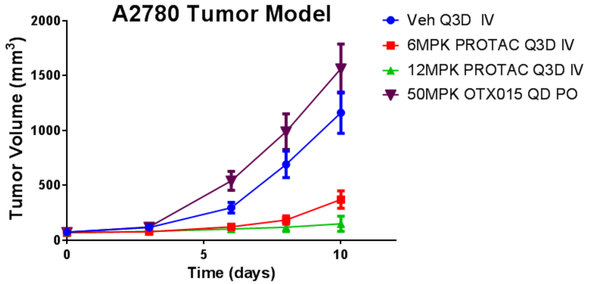


PROTACs are potent BRD4 degraders *in vivo*



- 8h following 1x IV dose
- Subcutaneous CDX model of DLBCL

PROTAC treatment is efficacious in an A2780 subcutaneous xenograft mouse model



Summary

- We have developed PROTACs that are potent BRD4 degraders in ovarian cancer cell lines and in tumor xenografts
- BRD4 PROTACs are efficacious degraders in vitro and in vivo, and result in stasis in an A2780 tumor model following intermittent IV dosing
- Ovarian cancer lines show differential sensitivity to PROTAC mediated BRD4 degradation
- We have found a number of genes known to be associated with chemo-resistance and disease outcome in ovarian cancer to be differentially regulated in highly PROTAC sensitive cell lines
- We hypothesize that BCLxL has the potential to be a clinical biomarker, with low levels being predictive of tumor sensitivity to BRD4 degradation in ovarian cancer