Discovery and characterization of a p300-selective degrader demonstrates potent anti-tumor activity in preclinical models of prostate cancer

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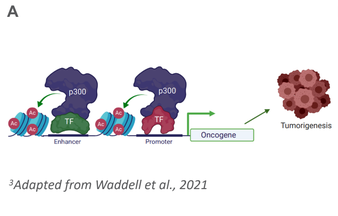
Background

- Although strategies targeting androgen receptor (AR) in the treatment of prostate cancer (PrCa) have shown clear clinical benefit[1], a variety of AR-signaling bypass mechanisms ultimately result in disease progression[2]
- The histone acetyltransferase paralogs p300 and CBP, key coactivators of AR, are currently under investigation as attractive therapeutic targets to abrogate aberrant AR signaling[3]
- To date, development of dual CBP/p300 inhibitors have faced challenges with clinical toxicity, as hematopoietic progenitors rely on these targets to maintain self renewal capacity[4]
- We postulate that p300 serves as a key regulator of AR signaling and by sparing CBP we should retain anti-tumor activity against AR-driven PrCa with an improved safety margin.

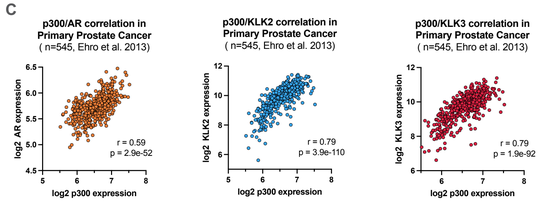
Key Findings

- Identified novel orally bioavailable p300-selective degraders
- p300 degradation inhibits the growth of AR+ prostate cancer
- Oral administration of p300 degraders demonstrated significant anti-tumor activity of AR+ prostate cancer CDXs in vivo

Introduction

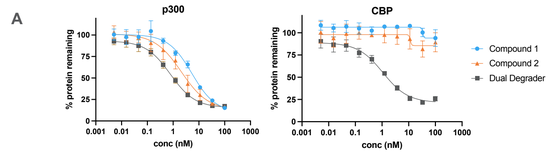


[3]*Adapted from Waddell et al., 2021*

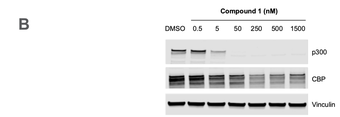


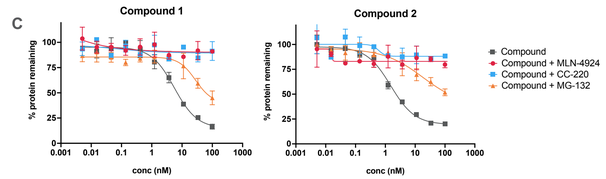
(**A**) Graphic demonstrating the proposed mechanism by which p300 can drive oncogenic processes. p300 can function as a coactivator of several transcription factors by acetylating histones, relaxing chromatin and allowing for the transcription of oncogenic drivers. (**B**) Cartoon depicting the cofactor role of p300 in androgen receptor signaling in prostate cancer, whereby p300 can acetylate AR, resulting in the association of the p300/AR complex to androgen response elements and subsequent transcription of AR target genes. (**C**) Tumor RNASeq from 545 patients with primary prostate cancer showing a high correlation between p300 expression and AR (orange), as well as two known AR target genes, KLK2 (blue) and KLK3 (red).

Figure 1. p300 degraders show superb selectivity and potency in vitro



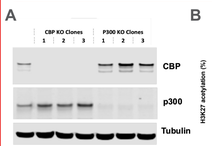
	P300		CBP	
	D_{max} (%)	DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)
Compound 1	85	6.5	13	> [max]
Compound 2	85	2.0	27	> [max]
Dual Degrader	84	1.0	79	1.5

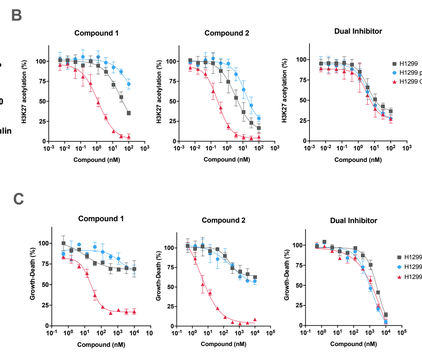




(**A**) Selectivity of degradation measured by HiBit knock-in of either p300 (left) or CBP (right) in A549 cells. (**B**) Dose response by Western blot following 6h of incubation shows selectivity for p300 in H1299 cells. (**C**) Degradation of p300 is dependent on the UPS system as evidenced by pretreatment with inhibitors of neddylation (MLN-4924, 1μM), cereblon (CC-220, 1μM) or the proteasome (MG-132, 1μM).

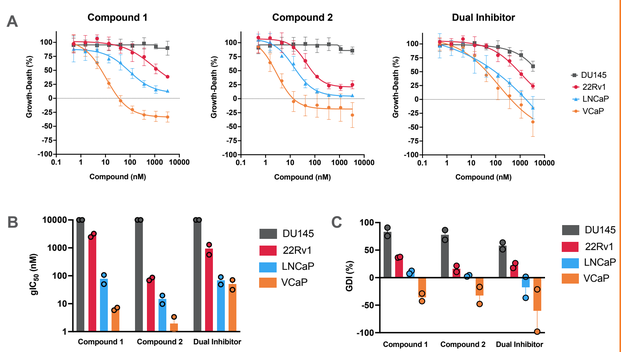
Figure 2. p300 degradation results in selective pharmacology using engineered model systems





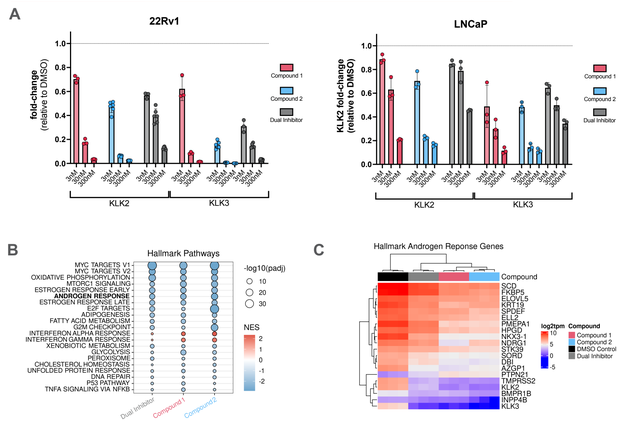
(**A**) Evidence of CRISPR-mediated knockout of p300 or CBP in H1299 cells. (**B**) Dose-dependent decreases in H3K27Ac observed in CBP KO cells by in-cell Western reflect the downstream pharmacology of degrading p300. (**C**) p300 degradation results in reduced cell viability in the context of CBP knockout.

Figure 3. AR+ prostate cancer cells lines exhibit sensitivity to p300-specific degraders



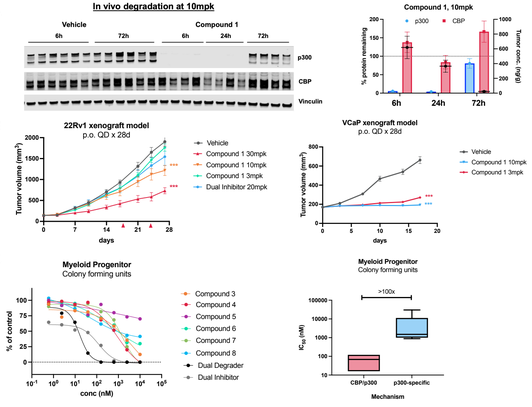
(**A**) Viability dose response curves of AR+ prostate cancer cell lines (22Rv1, LNCaP and VCaP) demonstrate sensitivity to p300 degraders, whereas AR- DU145 cells are insensitive. (**B**) Growth IC50 waterfall plots comparing p300 degraders to a dual p300/CBP inhibitor across PrCa lines. (**C**) Growth/death index waterfall plot showing cell growth inhibition (positive GDI) and cell death (negative GDI) across PrCa lines.

Figure 4. Degradation of p300 results in a suppression of AR-mediated gene signatures



(**A**) 22Rv1 and LNCaP prostate cancer cells were treated with increasing concentrations (3, 30, 300nM) of p300 selective degraders or a dual p300/CBP inhibitor for 48h. Changes in KLK2 and KLK3 mRNA expression were assessed by TaqMan PCR relative to the geomean of two housekeeping genes. (**B**) GSEA dot plot of 22Rv1 cells treated with 300nM of compound for 24h, comparing a dual inhibitor to p300 selective degraders. (**C**) Heat map of differentially expressed genes within the androgen response signature in DMSO and compound treated samples.

Figure 5. Oral administration of Compound 1 shows favorable pharmacodynamics, efficacy and therapeutic window



(**A**) Once daily oral administration of Compound 1 at 10mpk demonstrated significant and selective degradation of p300 in H1704 tumor xenografts assayed by Western blot. (**B**) Daily oral administration of Compound 1 resulted in significant tumor growth inhibition in the 22Rv1 and VCaP CDX models (*** p < 0.001, FDR < 0.01; ▲ mouse removed due to cachexia) (**C**) Several p300 selective degraders were compared to a dual degrader and inhibitor in an ex vivo myeloid progenitor colony forming assay where they exhibited significantly less toxicity.

Conclusions

- We identified selective orally bioavailable degraders with < 10 nM potency against p300
- CBP KO cells confirmed the on-target pharmacology of targeting p300 via selective H3K27 acetylation and growth inhibition
- p300 degradation inhibits the growth of AR+ prostate cancer cells in vitro and results in potent downregulation of AR target genes
- Oral administration of our compound demonstrated >90% degradation of p300 in vivo
- Dose levels shown led to significant anti-tumor activity in AR+ prostate cancer models in mice

1. Kim TJ, Lee YH and Koo KC. Current Status and Future Perspectives of Androgen Receptor Inhibition Therapy for Prostate Cancer: A Comprehensive Review *Biomolecules* **2021** 11(4): 492.

2. Watson PA, Arora VK and Sawyers CL. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. *Nat Rev Cancer* **2015** 15(12): 701-711.

3. Waddell AR, Huang H and Liao D. CBP/p300: Critical Co-Activators for Nuclear Steroid Hormone Receptors and Emerging Therapeutic Targets in Prostate and Breast Cancers. *Cancers* **2021** 13(12) 2872.

4. Rebel VI, Kung AL, Tanner EA, Yang H et al. Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. *PNAS* **2002** 99(23): 14789-14794.