IDENTIFICATION OF SELECTIVE CBP DEGRADERS WITH ROBUST PRECLINICAL PK/PD, EFFICACY AND SAFETY ACROSS SOLID TUMOR INDICATIONS

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ABSTRACT

CREB binding protein (CBP) and E1A binding protein p300 (EP300) are paralog histone acetyltransferases involved in many cellular processes via their activity as transcription factor co-activators. Dysregulation of one or both proteins has been implicated in various cancer types, and functional genomic screens have revealed a bidirectional synthetic lethal relationship between these two paralogs in tumor cells. Due to the high homology between CBP and EP300, identifying selective chemical matter that selectively disrupts the activity of CBP has proven challenging. Small molecule inhibitors targeting the HAT or bromodomain of CBP/EP300 have been developed; however, these agents exhibit hematopoietic toxicity resulting from dual inhibition, which limits their therapeutic window.

Herein, we describe the PK, PD, and efficacy of selective, potent CBP degraders across various EP300-mutant cancer xenograft models. Our results show deep and sustained CBP degradation, leading to significant tumor growth inhibition in solid tumors. This anti-tumor activity was not associated with significant body weight loss or hematopoietic toxicity. Our CBP-selective protein degraders have the potential to be a first-in-class therapeutic option for patients with tumors harboring EP300 mutations.

Figure 1. Targeting CBP in EP300 mutant cancers

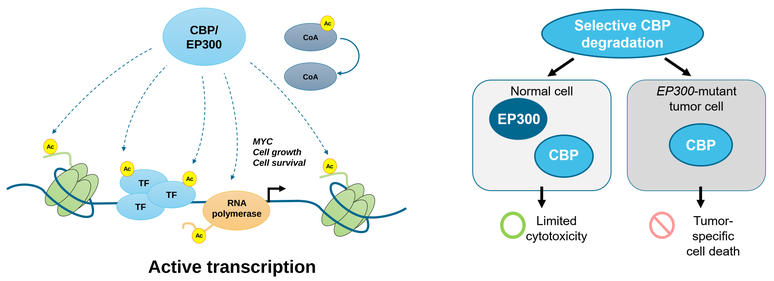
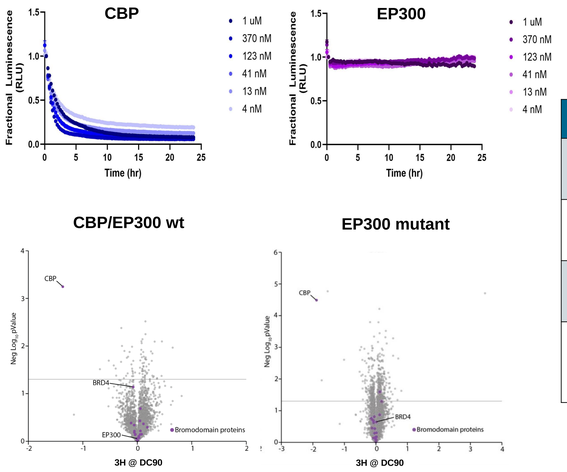


Figure 1: CBP/EP300 are histone acetyltransferases that function by regulating enhancer mediated transcription and protein stability. These paralogs have a bidirectional synthetic lethal relationship that can be exploited in a variety of cancer indications.

Figure 2. Identification of potent, selective CBP degraders



In Vitro Properties	dCBP-63
Cellular Potency DC_{50} (KI HiBiT)	>30 µM (EP300) **0.043 µM (CBP)**
Dmax (KI HiBiT)	0% (EP300) **90% (CBP)**
Kinetic Degradation Rate CBP (l) (hr-1)	**1.0**
Cellular Proliferation GI50 (µM) – RKO (EP300 mutant)	**0.02**

Figure 2: We have identified potent and selective degraders of CBP. Dose response kinetic data confirm CBP selectivity, with minimal effects on EP300 and other bromodomain containing proteins.

Figure 3. CBP selective degraders show an antiproliferative effect in EP300 mutant versus wild-type cell lines across indications

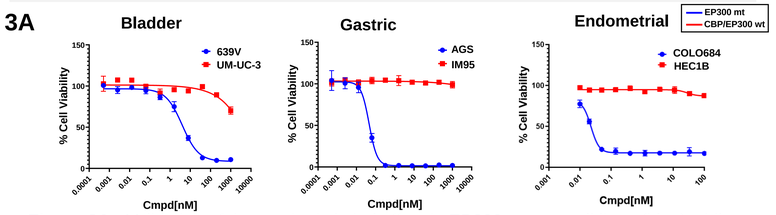


Figure 3A: Validation of the synthetic lethal effect in EP300 mutant cell lines (blue) while sparing the CBP/EP300 wild-type lines (red) across multiple indications in a 7 day CTG assay.

Figure 3B: Validation of the genetic CBP dependency using our selective degrader across many cell lines from multiple indications of interest.

Figure 4. In vivo PK/PD selectivity and efficacy

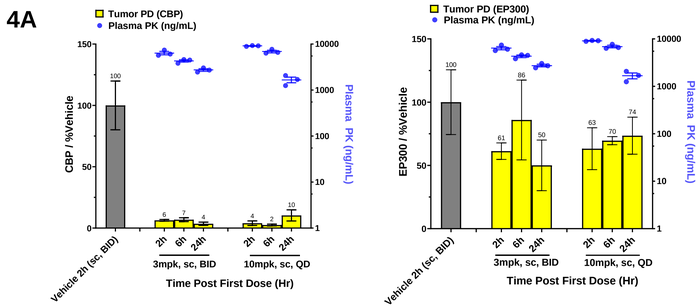


Figure 4A: Our in vivo enabled CBP selective degrader, dCBP-59, maintains similar degradation selectivity in vivo as observed in our in vitro assays.

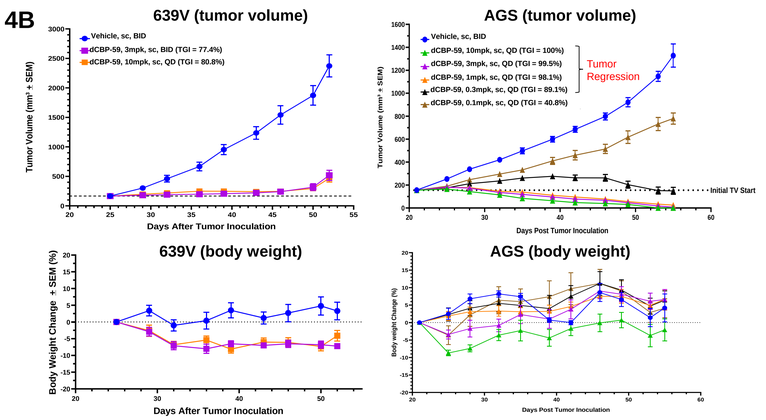


Figure 4B: We have achieved excellent efficacy in a number of different EP300 mutant xenograft models across multiple indications.

Figure 5. Degrader impact on H3K27Ac mediated transcription

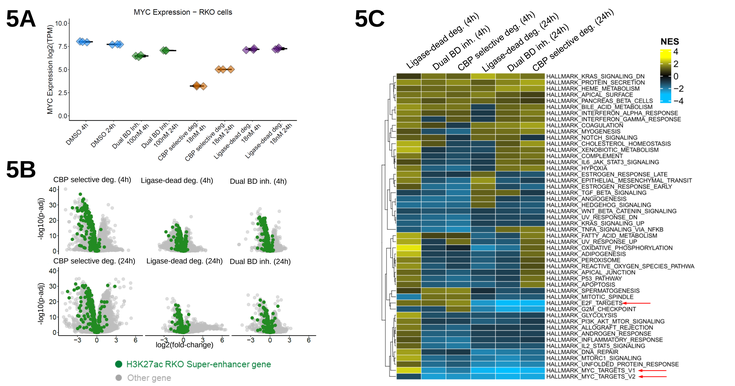


Figure 5: RNA-seq analysis shows down-regulation of (A) c-Myc and (B) RKO super-enhancer genes (defined by H3K27ac) after CBP degradation C) GSEA shows MYC and E2F targets down-regulated at 24 hours after CBP degradation.

Figure 6. Thrombocytopenia focused safety studies

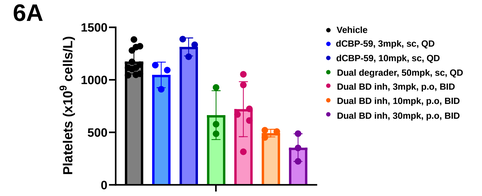


Figure 6A: Selective CBP degrader has no impact on total platelet counts compared to dual bromodomain inhibition following a 14 day mouse in vivo study.

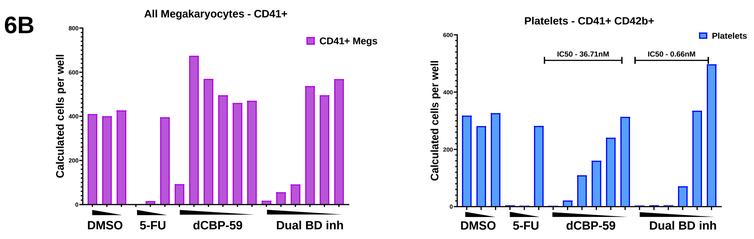


Figure 6B: In vitro megakaryocyte differentiation and platelet measurement assay confirms increased safety with a selective degrader over dual bromodomain inhibition.

CMPD	EP300 Bromo Binding [uM]	CBP Bromo Binding [uM]
Dual BD inh	0.001	0.001
dCBP-59	0.050	0.008

CONCLUSIONS

- We have generated potent and selective CBP degraders that validate the synthetic lethal relationship and show anti-tumor activity across various EP300 mutant cell lines from multiple indications.
- Using our in vivo enabled compounds we have achieved efficacy in a number of different EP300 mutant xenograft models, across multiple indications of interest.
- We have identified and are currently expanding our understanding of the mechanism of action and biomarkers of selective CBP degradation.
- We show that selective degradation of CBP results in a lower risk of thrombocytopenia as compared to dual CBP/EP300 bromodomain inhibitors.

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