Discovery and characterization of a p300-selective degrader with potent anti-tumor activity in CBP mutant cancers

Mike Russell, Harshil Dhruv, Cassandra L. Lowenstein, Xuqing Zhang, Jeremy Roach, Jianing Song, Brian Vidal, Rakesh Nagilla, Nathan Kendsersky, Matt Tudor, Qiaolin Deng, Clemente Aguilar-Bonavides, Elham Behshad, Sudeep Banjade, Zhihua Sui, Corey Strickland, Larry J. Jolivette, Helai P Mohammad

SK Life Science Labs, King of Prussia, PA; contact: mrussell@sklslabs.com

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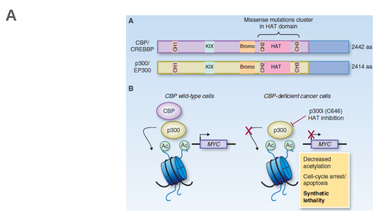
Background

- In efforts to expand the druggable genome while maintaining an emphasis on genetically targeted therapies, recent attempts have focused on exploiting synthetic lethal relationships
- Paralogous protein pairs are particularly compelling targets in this context due to the clear mechanistic basis for synthetic lethality - highlighted by recent work targeting the histone acetylase transferase p300 in the context of CBP-deficient cancers[1]
- 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[2]
- We postulate that p300-selective degradation in the context of CBP-deficient cancers should provide anti-tumor activity with an improved safety margin over dual mechanisms

Key Findings

- Identified novel orally bioavailable p300-selective degraders
- p300 degradation inhibits the growth of cancers with CBP LoF mutations
- Administration of selective degraders in vivo demonstrated significant degradation of p300 but not CBP

Introduction



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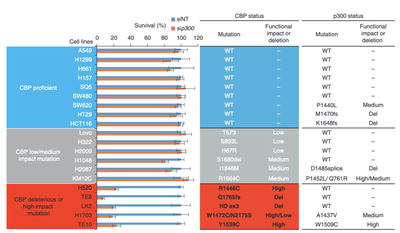
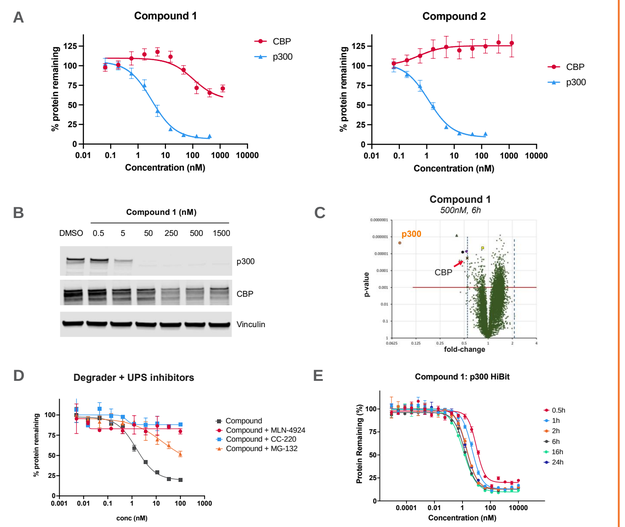


2015 Ogiwara et al.

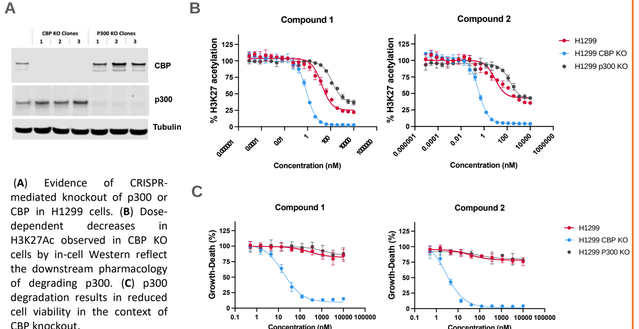
(**A**) Graphic depicting the high protein homology between CBP/p300 (top) and the synthetic lethal relationship between the two proteins (bottom). (**B**) Cell line data depicting the sensitivity to p300 depletion in the context of CBP loss-of-function mutations.

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



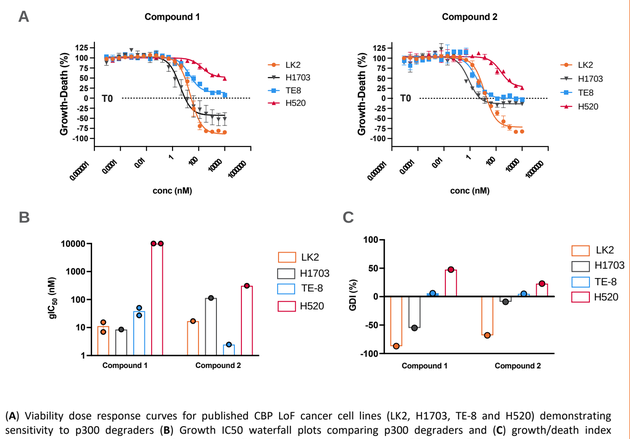
(**A**) Selectivity of degradation measured by HiBit knock-in of either p300 or CBP in A549 cells. Compound 1: $AbsDC_{50}$ = 3.2nM, Dmax = 90%; Compound 2: $AbsDC_{50}$ = 1.2nM, Dmax = 87%. (**B**) Dose response by Western blot following 6h of incubation shows selectivity for p300 in H1299 cells. (**C**) Global proteomics illustrates the selectivity in H1299 cells. (**D**) 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). (**E**) Kinetics of p300 degradation as measured by HiBit assay.

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



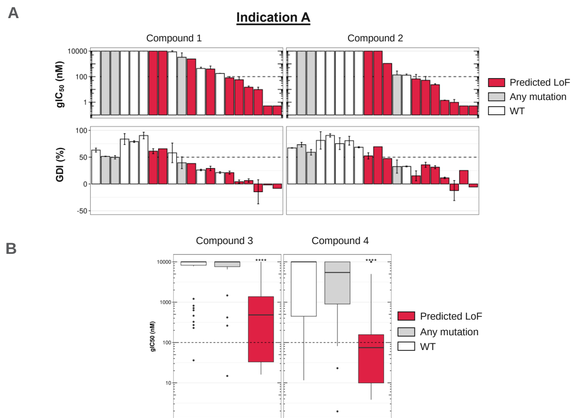
(**A**) Evidence of CRISPR-mediated knockout of p300 or CBP in H1299 cells. (**B**) Dose-dependent decreases in H3K27Ac observed in CBP KO cells by in-cell Western reflect the downstream pharmacology of degrading p300. (**C**) p300 degradation results in reduced cell viability in the context of CBP knockout.

Figure 3. Cancer cell lines harboring CBP LoF mutations exhibit sensitivity to p300-specific degraders



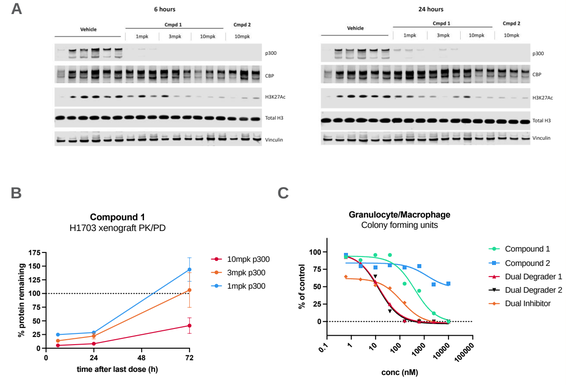
(**A**) Viability dose response curves for published CBP LoF cancer cell lines (LK2, H1703, TE-8 and H520) demonstrating sensitivity to p300 degraders (**B**) Growth IC50 waterfall plots comparing p300 degraders and (**C**) growth/death index waterfall plot showing cell growth inhibition (positive GDI) and cell death (negative GDI) across CBP LoF lines.

Figure 4. Degradation of p300 results in selective cell growth inhibition in predicted LoF cell lines across indications



(**A**) A broad cellular panel of a particular cancer subtype illustrating the sensitivity of cells with putative LoF mutations in CBP demonstrating the antiproliferative effects of p300-selective degraders. Notably, cell lines with CBP mutations that were not deemed LoF were insensitive to p300 degradation. (**B**) Growth IC50 of p300 selective tool compounds across a variety of cancer cell types harboring either predicted CBP LoF or other CBP mutations illustrating the sensitivity of the LoF mutant cells to p300 degradation.

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



(**A**) Once daily oral administration demonstrated significant and selective degradation of p300 in H1703 tumor xenografts assayed by Western blot. (**B**) Quantification of p300 degradation by Compound 1 in H1703 tumors over time show a dose-dependent persistence of degradation. (**C**) Selective p300 degraders were compared to dual degraders and a dual inhibitor using an ex vivo myeloid progenitor colony forming assay where they exhibited significantly less toxicity.

Conclusions

- We identified selective orally bioavailable degraders with < 5 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 cancer cells harboring known CBP loss-of-function mutations in vitro
- Oral administration of our compound demonstrated >90% degradation of p300 in vivo
- p300 selective degraders demonstrate significantly less toxicity than dual p300/CBP abrogation in preclinical hematopoietic progenitor assays

1. Ogiwara H, Sasaki M, Mitachi T et al. Targeting p300 Addiction in CBP-Deficient Cancers Causes Synthetic Lethality by Apoptotic Cell Death due to Abrogation of MYC Expression *Cancer Discovery* **2016** Apr;6(4):430-45.

2. 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.

3.Kadoch C. Lifting Up the HAT: Synthetic Lethal Screening Reveals a Novel Vulnerability at the CBP-p300 Axis. *Cancer Discovery* **2016** Apr;6(4):350-2.