#5163: Leveraging selective degradation of CBP and EP300 for potent anti-cancer activity

Karolina Mizeracka, Benjamin Adams, Ketaki Adhikari, Hafiz Ahmad, Brandon Antonakos, Wesley Austin, Mike Collins, Danette Daniels, Julie Di Bernardo, Thomas Dixon, Claudia Dominici, Brian Ethell, Katharine Feldman, Md Imran Hossain, Laura La Bonte, Dave Lahr, Janice Lee, Michael Lehrke, Mei Yun Lin, David Mayhew, Paige Monsen, Solymar Negretti, John Pulice, Abira Ramakrishan, Dipti Sadalge, Darshan Sappal, Shawn Schiller, Gromek Smolen, Nihan Ucisik, Laura Von der Porten, Jessica Wan, Grace Werosta, Molly Wilson, Elizabeth Wittenborn, Qianhe Zhou, Mark Zimmerman, Steven Bellon

Foghorn Therapeutics, 99 Coolidge Ave., Watertown, MA 02472

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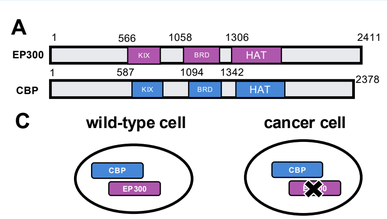
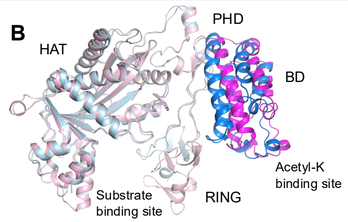
Introduction

Numerous cancers have shown dependency on one of two paralog histone acetyltransferases, CREB binding protein (CBP) or E1A binding protein (EP300). For example, EP300-mutant gastric and colorectal malignancies strongly depend on CBP function for growth and survival. Here we describe our platform to develop fast, potent, and selective CBP and EP300 degraders that remove only one of the targets, while sparing the activity of its paralog. Treatment with our selective CBP degrader results in potent anti-cancer activity in EP300-mutant cancers, while maintaining a wider therapeutic window than dual inhibition.

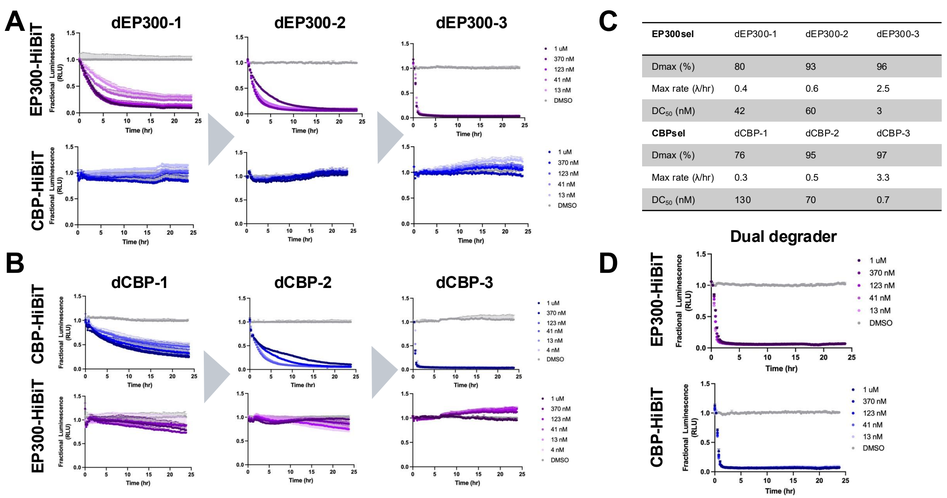




CBP and EP300 are very similar paralogs:

- HAT domains are 88% identical
- Bromodomains are 97% identical
- Known binders/inhibitors are not selective and have tolerability issues

Discovery of CBP and EP300 dual and selective degraders



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EP300sel	dEP300-1	dEP300-2	dEP300-3
Dmax (%)	80	93	96
Max rate (λ/hr)	0.4	0.6	2.5
DC_{50} (nM)	42	60	3
CBPsel	dCBP-1	dCBP-2	dCBP-3
Dmax (%)	76	95	97
Max rate (λ/hr)	0.3	0.5	3.3
DC_{50} (nM)	130	70	0.7

Fig 1. Degrader compounds are triaged through live-cell 24 hr kinetic degradation assays using CBP- and EP300-HiBiT cell lines. Compounds are optimized for potency, high Dmax, and fast rates of on-target degradation, while maintaining minimal off-target degradation (A, B, C). A fast and potent dual degrader was characterized in the kinetics assay as a control (D).

Ternary complex profiles of selective and dual degraders

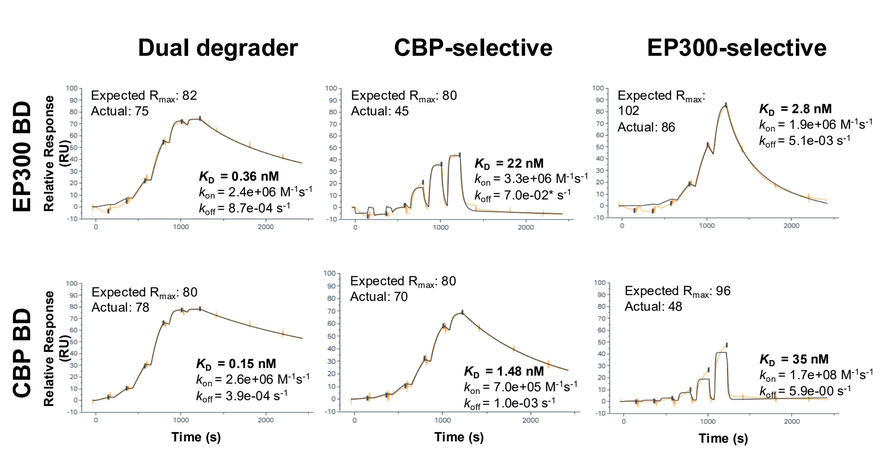

Fig 2. Dual and selective degraders were tested for ternary complex formation by SPR with VHL immobilized on the sensor chip. Compounds were incubated with an excess of CBP or EP300 BD and flowed over the chip. On and off rates were determined using a Langmuir 1:1 binding model.

Structural insight into ternary complex formation

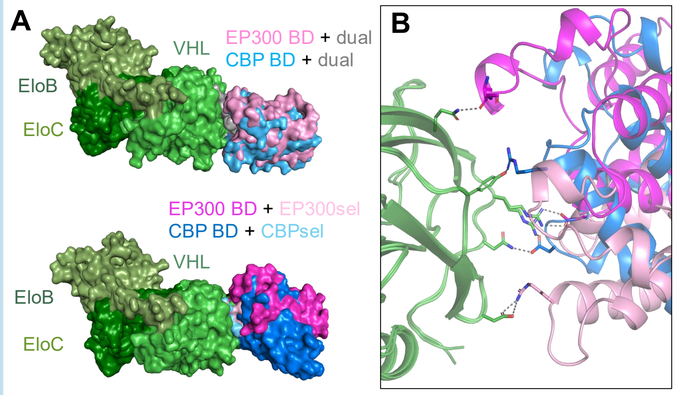

Fig 3. X-ray crystal structures of CBP bromodomain (blue) or EP300 bromodomain (pink) interacting with VHL (green) in complex with CBP-selective, EP300-selective, or dual degraders. Different degrader selectivities correlate with different ternary complex orientations. The orientation of the target protein could contribute to differential ubiquitination, leading to selective degradation.

Selective target ubiquitination

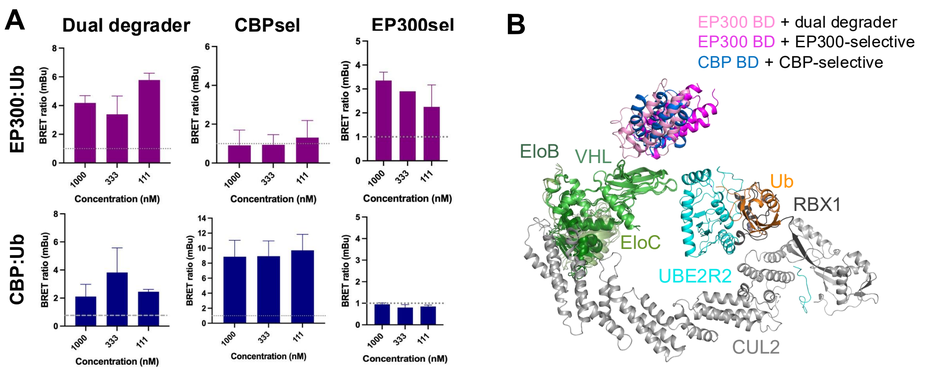

Fig 4. Levels of ubiquitination induced by compound treatment detected through NanoBRET proximity assays. Luminescent donor – CBP:HiBiT or EP300:HiBiT; fluorescent acceptor - HaloTag antibody conjugated to ubiquitin. Cells were treated for 90 min with varying concentrations of a dual degrader or a CBP-selective degrader with CBP and EP300 as targets (A). Cryo-EM structure of $CRL2^{VHL}$-UBE2R2-MZ1-BRD4BD2 (PDB ID 8R5H) used to model $CRL2^{VHL}$-E2 complexes with the CBP and EP300 BD ternary crystal structures. Structures were aligned by VHL (B).

CBP degradation results in strong TGI in EP300-mutant models

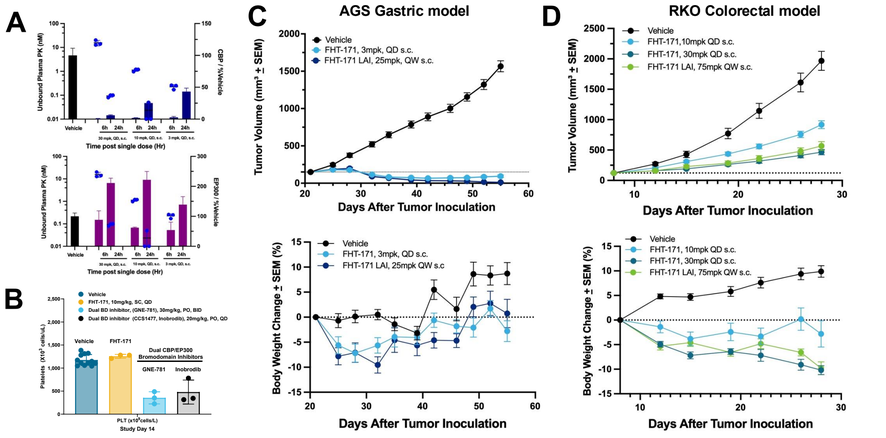

Fig 5. Single subcutaneous dose of lead selective CBP degrader FHT-171 in MCF7 xenograft-bearing mice results in significant CBP but not EP300 degradation (A). Treatment with FHT-171 spares platelet counts as compared to dual inhibitors (B). Tumor volume and body weight changes in EP300-mutant models (AGS, C and RKO, D) xenograft-bearing mice treated with FHT-171.

Conclusions

- We have developed fast, potent, and highly selective CBP and EP300 heterobifunctional degraders
- Selective, but not dual, degraders induce selective target ubiquitination in cell-based assays, which likely arises due to differential position of the BD domain relative to the Cullin ring VHL complex
- Our selective CBP degraders maintain selectivity of degradation over EP300 *in vivo*
- Treatment with our lead selective CBP degrader in EP300-mutant *in vivo* models leads to strong tumor growth inhibition without any adverse effects on platelet counts