Abstract #1643

ESTABLISHING RATIONAL COMBINATION STRATEGIES WITH SELECTIVE CBP DEGRADERS IN SOLID TUMOR INDICATIONS

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ABSTRACT

The paralog lysine acetyltransferases CREB binding protein (CBP) and E1A binding protein P300 (EP300) function as transcriptional coactivators that regulate diverse cellular programs. Mutations in *CBP* and *EP300* have been implicated in the biology of various cancer types. The bidirectional synthetic lethal relationship between these paralogs in tumor cells highlights a therapeutic opportunity in targeting CBP selectively in *EP300*-mutant cancers. We have previously demonstrated that our selective CBP degraders have potent antiproliferative activity in *EP300*-mutant cancers through both in vitro assays and cell line-derived xenograft models when used as a single agent. We have now expanded our studies to explore strategies for combination treatment with approved chemotherapies to enhance tumor growth inhibition.

Here, we demonstrate that our selective and potent CBP degrader compounds provide combinatorial benefit when used with approved chemotherapeutic and targeted agents. Through a large-scale in vitro combination screen, we show that CBP degrader treatment synergizes with multiple standard-of-care chemotherapies, including paclitaxel, as well as targeted agents, including CDK4/6 inhibitors. Furthermore, by genome-wide CRISPR knockout screening across four cell line models of different solid tumor types, we establish genetic determinants of resistance and sensitivity to CBP degradation in an *EP300*-mutant background, providing further mechanistic insight into combination therapy approaches. Collectively, we have defined cellular processes that present therapeutic vulnerabilities that can be leveraged for rational combination strategies with selective CBP degraders in solid tumor indications.

CBP degraders are potent and selective

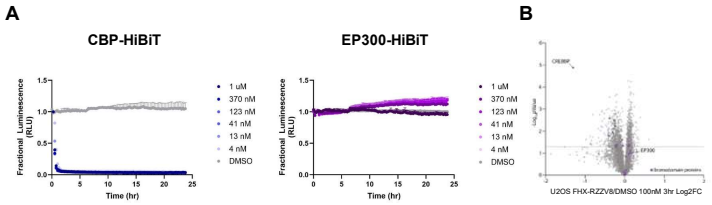


Figure 1. A) Dose-response kinetic profiling of CBP degrader FHX-RZZV8 confirms potency and selectivity of CBP degradation over EP300. **B)** Proteomics demonstrates selectivity of FHX-RZZV8 for CBP over other proteins, including those containing bromodomains.

Genome-wide CRISPR screening identifies sensitizing targets in cell cycle related genes upon CBP degradation

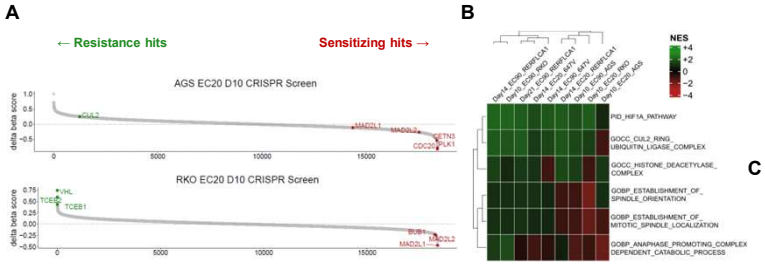


Figure 2. CRISPR screen of *EP300*-mutant cell lines over 8-10 cell doublings during FHX-LX4CF treatment **A)** Sensitivity (beta) scores between DMSO and CBP degrader conditions identify sensitizing/resistance genes **B)** GSEA of the delta beta scores identify gene sets of VHL components and deacetylases as enriched for resistance hits and cell cycle and mitotic spindle genes enriched in sensitizing hits.

RESULTS

High-throughput screening with CBP degrader reveals synergistic combination agents

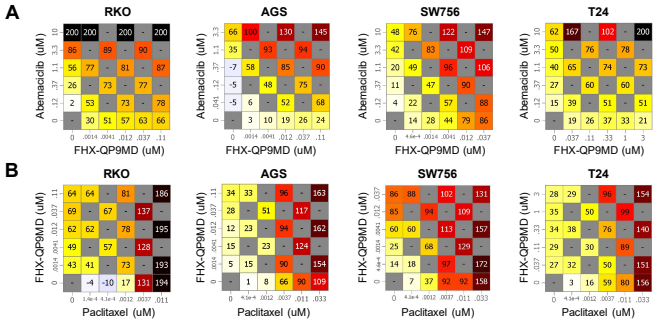


Figure 3. Checkerboard matrices display percent growth inhibition in EP300-mutant cell lines treated with a combination of CBP degrader FHX-QP9MD and **A)** CDK4/6 inhibitor abemaciclib or **B)** paclitaxel for 6 days.

PRISM screening and DepMap suggest indications for CBP degraders beyond *EP300*-mutant cancers

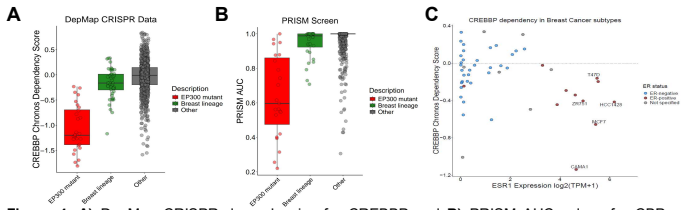
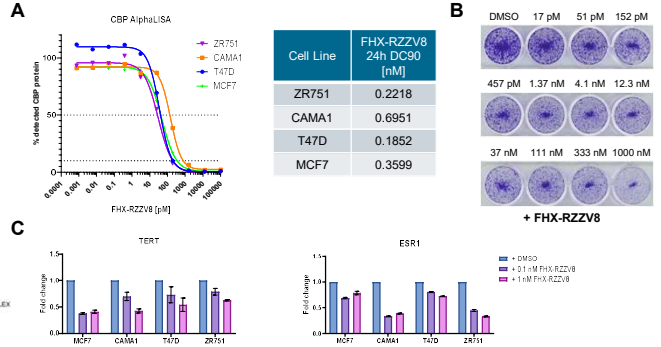


Figure 4. A) DepMap CRISPR dependencies for CREBBP and **B)** PRISM AUC values for CBP degrader FHX-RZZV8 include *EP300*-mutant lines but other cell lines are sensitive, not explained by *EP300* status. **C)** ER+ breast cancer cell lines demonstrate sensitivity to CBP loss.

CBP degraders demonstrate activity in ER+ breast cancer



Cell Line	FHX-RZZV8 24h DC90 [nM]
ZR751	0.2218
CAMA1	0.6951
T47D	0.1852
MCF7	0.3599

Figure 5. A) CBP AlphaLISA characterization of CBP degrader FHX-RZZV8 in ER+ breast cancer cell lines after 24 hours of treatment. **B)** Crystal violet staining of MCF7 cell line colonies after 14 days of treatment with FHX-RZZV8. **C)** qPCR in ER+ breast cancer cell lines after 7-day treatment with FHX-RZZV8. Error bars indicate SEM.

CBP degrader treatment with CDK4/6 inhibition provides combinatorial benefit in ER+ breast cancer

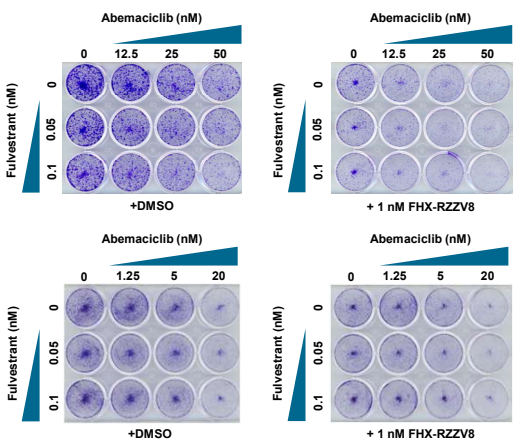


Figure 6. Crystal violet staining of **A)** MCF7 and **B)** T47D cell line colonies after 14 days of treatment with abemaciclib, fulvestrant, and DMSO or 1 nM FHX-RZZV8 indicates combinatorial benefit of triplet therapy. **C)** Proposed model by which CBP controls proliferative programs in ER+ breast cancer.

CONCLUSIONS

- Genome-wide CRISPR screening with selective CBP degrader treatment indicates that disruption of cell cycle and mitotic spindle assembly regulators sensitizes cells to CBP degradation
- Combination screening with CBP degraders highlights synergistic combination partners, including paclitaxel and abemaciclib
- PRISM screening suggests activity in indications beyond *EP300*-mutant cancers for selective CBP degraders
- CBP degrader treatment has antiproliferative activity in ER+ breast cancer cell lines and downregulates genes involved in tumorigenic programs
- Triplet therapy with a CBP degrader, abemaciclib, and fulvestrant provides combinatorial benefit in ER+ breast cancer cell lines