CBP-8008: A first-in-class targeted pan-Bet protein degradation therapy using bi-specific XDC (Bi-XDC) conjugate for TNBC and mCRPC

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COHERENT BIOPHARMA

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Introduction

PROTAC (PROteolysis-TArgeting Chimeras) induces protein degradation via an "event-driven" pharmacology, and provides a comprehensive and sustained suppression of target proteins. Consequently, PROTAC presents a paradigm shift by addressing previously undruggable or challenging targets such as KRAS and c-Myc *et al*, and has attracted significant attention from both academia and pharmaceutical industries.[1] However, poor bioavailability, and lack of cellular targeting result in low efficacy and possible off-target toxicity. Various deliver systems, such as prodrugs, peptide or antibody based conjugates have been invented to overcome these limitations, and great progress have been made in recent years.[2]

Bi-XDC (Bi-specific-ligand drug conjugate) is a pioneering deliver system developed by Coherent Biopharma, enabling payloads with improved bioavailability, and cell selectivity. The synergy from bi-ligands enhances Bi-XDC's affinity and capability to overcome the competition from endogenous ligands. Bi-XDC penetrates into tumor cells much faster than antibody, and is enriched and sustained for more than 5 days in the receptor expression positive tumor models.

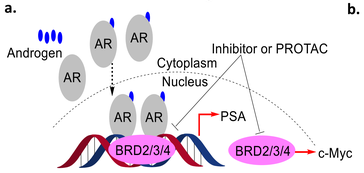
Herein, we report a PROTAC conjugate, CBP-8008, a pan-BET degrader with a bi-specific-ligand module, which enables PROTAC with tumor cell selectivity and penetration capabilities. After screening a panel of cancer cell lines and CDX/PDX models, it shows potential in the treatment of TNBC and mCRPC.

Design of CBP-8008

The BET family (BRD2, BRD3, BRD4, and BRDT) proteins regulates numerous immunity and cancer-related genes and pathways, such as c-Myc and androgen receptor (AR) (**Figure 1a**).[3] Dysfunction of BET proteins, specifically BRD4, associates closely with the development and progression of TNBC and mCRPC (**Figure 1b**).[4]

Folate receptor alpha (FRα) is highly expressed in many types of cancers to support the rapid proliferation and growth.[5] Prostate Specific Membrane Antigen (PSMA) is related with PI3K/Akt activation and uptake of folate,[6] highly expressed in prostate cancer and TNBC. Clinical results of CBP-1018 proves the pair of FRα/PSMA receptors on treatment of prostate cancer.[7] So FRα/PSMA bi-ligand is chosen delivering pan-BET PROTAC for treatment of mCRPC and TNBC. The proposed MOA of CBP-8008 is shown as **Figure 2**.

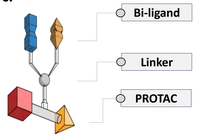
a.



b.

Receptor expression	mCRPC	TNBC
FRα	4.8%	59.5%
PSMA	99.8%	44.0%
Pan-BET	100%	100%

c.



- Bi-ligand: bind to FRα and PSMA, and deliver PROTAC into cells via receptor-mediated endocytosis (RME).
- Linker: tune physical properties, such as PK profiles, release site and rate.
- PROTAC: degrade target proteins.

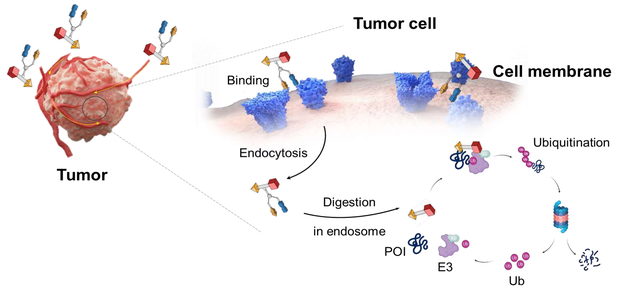


Figure 2. Proposed Mechanisms of Action of CBP-8008[8]

Results and Discussions

1. CBP-8008 degrades proteins via UPS

CBP-8008 degraded BRD4 and other BET proteins of TNBC cells, and led to the downregulation of c-Myc accordingly (**Figure 3a**, **3b**), by hijacking the Ubiquitin-Proteasome System (UPS). In the presence of either MG132, POI or E3 ligand, degradation was blocked. (**Figure 3c**)

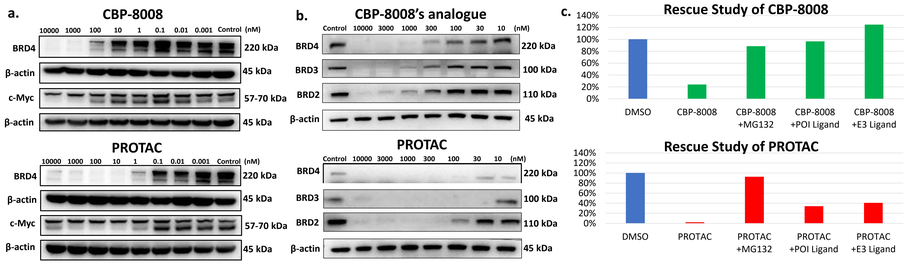


Figure 3. Study of CBP-8008 and PROTAC's function. **a**. CBP-8008 degrades BRD4 and downregulates c-Myc in MDA-MB-468 cells as PROTAC; **b**. CBP-8008's analogue, degrades pan-BET proteins, especially BRD4, as PROTAC; **c**. BRD4 Protein rescue experiment validates CBP-8008 degrades protein via UPS pathway, as PROTAC does. 10 nM of CBP-8008 and PROTAC were used respectively. 10 μM of MG132, POI ligand and E3 ligand were added to rescue degradation.

2. CBP-8008's efficacy comes from PROTAC

CBP-8008 released PROTAC via Cathepsin B mediated cleavage and exerted cell cytotoxicity. Concentration of PROTAC reached steady after 1 h and sustained more than 8 h. (**Figure 4a**)

By comparison, **CR202F2**, a non-cleavable linker connecting the same bi-ligand and PROTAC as CBP-8008, did not show any cytotoxicity due to no PROTAC released. (**Figure 4b**)

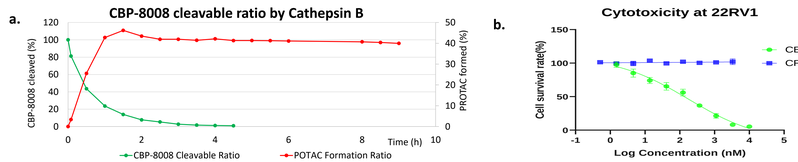


Figure 4. CBP-8008 released PROTAC and shows cytotoxicity. **a.** Incubation of CBP-8008 with Cathepsin B led to steady release of PROTAC after 1 h. No CBP-8008 was detected after 4.5 h while PROTAC's concentration kept till the ending of test; **b. CR202F2**, a conjugate as a negative control with a non-cleavable linker.

3. CBP-8008's PK/PD

After intravenous administration with the same molar dose of CBP-8008 and PROTAC in mice, CBP-8008 remained much higher concentration in plasma, while PROTAC was metabolized in 8 h. CBP-8008 prolonged the exposure of PROTAC and avoided potentially hematotoxicity. (**Figure 5a**)

At a FRα expression positive HT-29 CDX model, after single dosing the same molar equivalent of CBP-8008 and PROTAC, CBP-8008 and PROTAC released from CBP-8008 kept higher concentration in tumor than PROTAC. (**Figure 5b**)

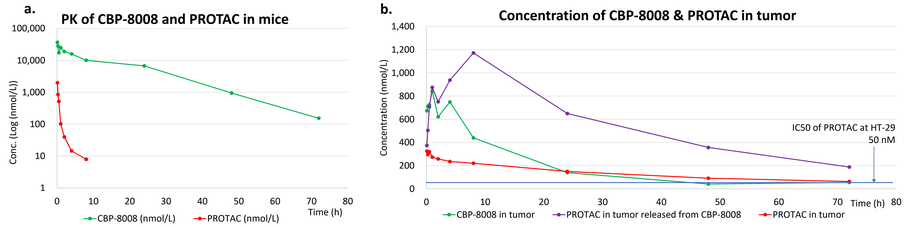


Figure 5. **a**. CBP-8008 extended the half life of PROTAC from 2.76 h to 9.12 h; **b**. Under the same molar equivalent dose (6.25 mg/kg for CBP-8008 and 2 mg/kg for PROTAC), PROTAC released from CBP-8008 was higher than dosed as PROTAC.

4. CBP-8008 reduced MED of PROTAC

Preliminary study indicated that PROTAC's MED was determined to be 4 mg/kg. In contrast, CBP-8008 demonstrated notable efficacy with as low as 1 mg/kg (equivalent of 0.32 mg/kg of PROTAC) in the same CDX model. (**Figure 6**)

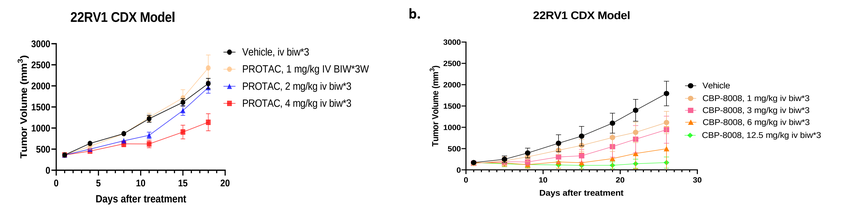


Figure 6. MED study of CBP-8008 and PROTAC on the same 22RV1 CDX model. **a**: MED of PROTAC was 4 mg/kg; **b**: MED of CBP-8008 was 1 mg/kg;

5. CBP-8008's in vitro efficacy depends on receptor expression and protein degradation

More efficacy studies in prostate cancer, TNBC, colorectal cancer and lung cancer cell lines was conducted. Both TNBC and prostate cancer cell lines were sensitive to CBP-8008. (**Figure 7a**)

PROTAC showed cytotoxicity to cancer cell lines sensitive to protein degradation (MDA-MB-231 and DU4475), while CBP-8008 only caused cytotoxicity to cell lines with both receptor expression positive and sensitive to protein degradation (MDA-MB-231). CBP-8008 had no effect to DU4475 since both folate and PSMA receptors are negatively expressed. If cell lines were insensitive to protein degradation (HCC70 and Caco-2), CBP-8008 had no response at all.

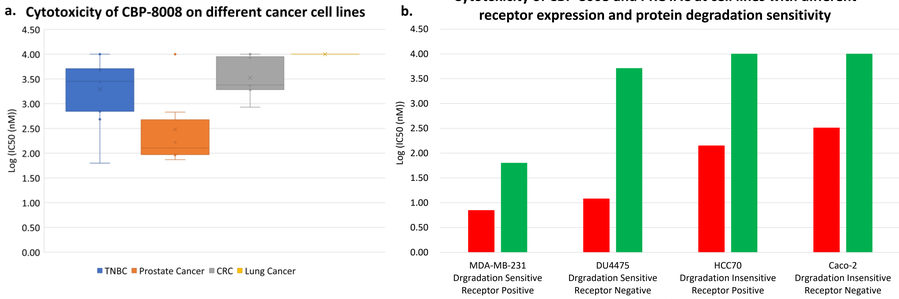


Figure 7. **a.** CBP-8008 showed potent cytotoxicities to TNBC and prostate cancer cells. **b.** Cytotoxicity of CBP-8008 and PROTAC in different receptor expression status and sensitivity to protein degradation.

6. CBP-8008 inhibits tumor growth in receptor expression positive models

Evaluation of CBP-8008 in prostate cancer, TNBC, ER positive breast cancer, ovarian cancer, colorectal cancer as well as pancreatic cancer at PDX and CDX models was done. Up to 90% tumor growth inhibition was observed, together with less than 5% body weight loss, indicating that CBP-8008 is a promising candidate for prostate cancer and TNBC treatment choice. In both cases, CBP-8008 performs better than PROTAC. (**Figure 8**)

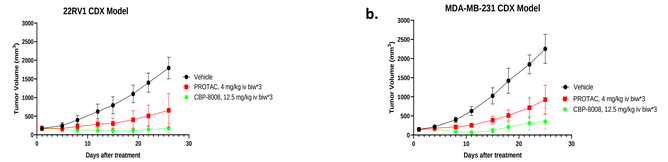


Figure 8. Tumor growth inhibition of CBP-8008 and PROTAC at CDX models.

Conclusions

1. Bi-specific ligand system enables PROTAC with cell selectivity and penetration, and better druggability, which can expand the advantages and minimize the disadvantages of PROTAC.
2. CBP-8008, an innovative bi-ligand (folic acid and PSMA) enabled pan-BET protein degrader, is being explored as a potential treatment for triple-negative breast cancer (TNBC) and metastatic castration-resistant prostate cancer (mCRPC). Preliminary preclinical assessment has validated its MOA, favorable safety profiles and efficacy in receptor-expression positive CDX/PDX models, particularly in TNBC and mCRPC models.

Reference

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