# Resistance to BET inhibitor leads to new therapeutic vulnerabilities in Castration-Resistant Prostate Cancer

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**Abstract**

BRD4 plays a major role in the transcription networks orchestrated by androgen receptor (AR) in castration resistant prostate cancer (CRPC) cells. Bromodomain and extraterminal protein (BET) inhibitors displace BRD4 protein from chromatin, resulting in the inhibition of oncogenic transcriptional programs. Several BET inhibitors (BETi) are currently being evaluated in clinical trials for a variety of malignancies, including CRPC. Here we describe a general mechanism of acquired resistance to BETi due to modulation of cellular pathways that are amenable to targeted therapies in CRPC cells. BETi resistant CRPC cells displayed cross-resistance to a variety of BETi in the absence of gatekeeper mutations or persistent drug pump activation. Resistant cells exhibited reduced chromatin bound BRD4, and were less sensitive to Proteolysis Targeting Chimeric (PROTAC) -mediated BRD4 degradation or genetic knockdown, suggesting a BRD4-independent transcription program. Transcriptomic analysis revealed reactivation of AR-signaling due to CDK9-mediated serine-81 phosphorylation of AR, with a consequent increase in sensitivity to CDK9 inhibitors and enzalutamide in BETi resistant cells. Additionally, increased DNA damage associated with PRC2-mediated transcriptional silencing of DNA damage response (DDR) genes was observed due to the loss of BRD4 from their proximal promoter regions in the resistant cells, leading to PARP inhibitor sensitivity. Collectively, our results identify the therapeutic limitation of BETi as a monotherapy in CRPC. However, data showing the reactivation of AR-signaling and increased DNA damage in the BETi resistant cells provide unique opportunities for combination therapies in managing CRPC. In conclusion, our study provides a strong rationale for therapies that include BETi in combination with CDK9 inhibitors, anti-androgen enzalutamide, PARP inhibitors, and platinum based drugs, up-front or in BETi refractory disease for CRPC patients in the clinic.

**Keywords:** BET inhibitors, acquired resistance, transcription, chromatin, AR, CDK9, BRCAness, PRC2 complex

Conflict of Interest: None

Funding Acknowledgement: This work was supported by a Department of Defense Idea Development award (W81XWH-17-1-0404) to I.A.A.