EZH2-mediated epigenetic underpinnings in advanced prostate cancer subtypes

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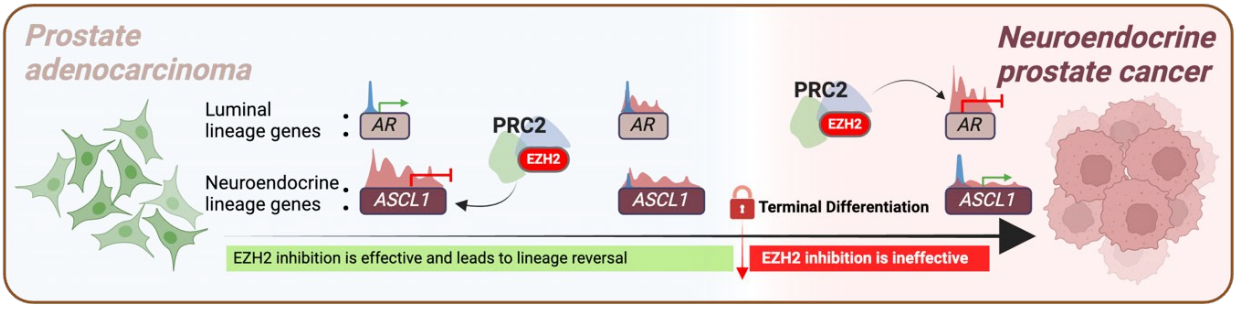

Background: Lineage plasticity with histologic transformation from prostate adenocarcinoma (PRAD) to neuroendocrine prostate cancer (NEPC) is a mechanism of treatment resistance. NEPC is associated with poor prognosis, and novel targeted therapies are urgently needed.
EZH2, a histone methyltransferase and emerging therapeutic target, is overexpressed in most treatment-resistant prostate cancers and is implicated as a driver of disease progression. Here, we define the differential, lineage-specific action of EZH2 in both PRAD and NEPC subtypes to better understand its role in modulating differentiation, lineage plasticity, and to identify mediators of response and resistance to EZH2 inhibitor therapy.

Methods: A panel of cell lines and patient-derived organoid/xenograft models representing PRAD (LNCaP and LNCaP-abl) and NEPC (WCM154, WCM155, WCM1262, WCM1078, WCM12) was employed to understand the response to the EZH2 inhibitor tazemetostat. Integrative RNA-Seq, CUT&RUN, and CUT&Tag were performed on PRAD and NEPC clinical samples and models to determine the influence of EZH2 in subtypes of prostate cancer.

Results: Compared to the robust response seen in PRAD models, our NEPC patient-derived models showed a modest response to EZH2i (tazemetostat) both *in vitro* and *in vivo*. While studies using PRAD-to-NEPC transitioning plasticity models have shown that EZH2i results in a reversal back towards a luminal lineage, we highlight that EZH2 inhibition in terminally differentiated NEPC models results in further maturation and forward differentiation of the neuroendocrine phenotype rather than a reversal in lineage. Epigenetic H3K27me3 CUT&Tag profiling of castration-resistant PRAD (n=9) and NEPC (n=9) clinical samples highlighted how PRC2 targets, including NE-lineage transcription factors, are de-repressed in NEPC. Mechanistically, EZH2 modulates bivalent genes, resulting in the upregulation of NEPC-associated transcriptional drivers (e.g., *ASCL1*) and neuronal gene programs, leading to forward differentiation after targeting EZH2 in NEPC. Interrogating the co-activator function of EZH2, we find that it is more prevalent in PRAD and nearly absent in NEPC, potentially due to the absence of AR in NEPC. Response to EZH2 inhibition may be mediated in

part by downstream effects on cell cycle target genes. We exploited the lack of EZH2-mediated regulation of cell-cycle-related genes in NEPC by using novel macrocyclic compounds targeting the cyclin/CDK complex, which are currently under clinical development, and highlight their potential to be explored further as a treatment strategy for NEPC.

Conclusion: EZH2i shows a modest response in NEPC preclinical models. Our results provide insights into a potential role for bivalent promoters in EZH2-mediated lineage reprogramming, which may facilitate forward differentiation in NEPC upon EZH2i. Subtype-specific downstream effects of EZH2 inhibition on cell cycle genes support the potential rationale for co-targeting cyclin/CDK to overcome resistance to EZH2 inhibition. This work informs ongoing EZH2 inhibitor clinical trials on patient selection in advanced prostate cancer.

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