Synergistic activity of tazemetostat in combination with androgen signaling inhibitors in preclinical models of prostate cancer demonstrates potential for clinical expansion

Vinny Motwani, Dorothy Brach, Liuye Huang, Chloe Pantano, Vanja Estanek, Jeffrey A. Keats, Lindsey U. Rodrigues, Kimberly Stickland, Daniel T. Dransfield, Alejandro Raimondi

Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States. Current available treatments include chemical castration and therapies targeting androgen receptor (AR) signaling pathways. Although initial responses are observed, 30% of patients have tumors that develop resistance to androgen deprivation therapy. Abiraterone (ABI) is a steroidogenesis inhibitor that competitively antagonizes the 17 β-hydroxysteroid dehydrogenase type 3 enzyme to reduce androgen synthesis, while enzalutamide (Enza) is a non-selective AR antagonist. Inhibitors of polycomb group protein 2 (EZH2) complex such as tazemetostat (Taz) are also potential antitumor agents in metastatic castration-resistant PCa (mCRPC) and AR translocation-positive prostate cancer (ATPC) models. Several studies have shown that EZH2 inhibition combined with abiraterone and enzalutamide improves clinical outcomes in mCRPC patients. In this study, we evaluated the potential of EZH2 inhibition in combination with androgen signaling inhibitors in preclinical models of prostate cancer.

**Methods and Materials**

Gene expression profile of PCa cell lines representing PCa subtypes in two models

**Results**

- Tazemetostat demonstrated modest antitumor activity as single agent but no benefit was observed in combination with Enza in 22Rv1 model
- Target engagement was observed in all groups containing tazemetostat in both the models tested

**Conclusions**

- EZH2 inhibition resulted in time- and dose-dependent antiproliferative activity in PCa cells that are dependent on AR signaling or are of the neuroendocrine subtype
- Treatment of PCa cell lines with tazemetostat in combination with ASIs resulted in synergistic cell growth inhibition that was driven by apoptotic cell death and G0/G1 cell cycle arrest
- Tazemetostat enhanced antitumor activity of ASIs in the LNCaP mouse xenograft model. However, in the 22Rv1 in vivo model (enzalutamide-resistant, AR-V7-positive) only tazemetostat as monotherapy displayed modest antitumor activity compared to all other treatments
- A significant decrease in intratumoral H3K27me3 levels was observed in both in vivo models by treatment with tazemetostat alone or in combination with ASIs indicating activity of tazemetostat
- In vivo PROTAC knockdown of AR-target genes showed that treatment with tazemetostat maintains the effect induced by ASIs in vivo
- These preclinical data supported the initiation of Phase 2/3 clinical trial for tazemetostat in combination with Enza in mCRPC

Abbreviations: AR = Androgen Receptor, Y = yes, N = no

**Abstract**

Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States. Current available treatments include chemical castration and therapies targeting androgen receptor (AR) signaling pathways. Although initial responses are observed, 30% of patients have tumors that develop resistance to androgen deprivation therapy. Abiraterone (ABI) is a steroidogenesis inhibitor that competitively antagonizes the 17 beta-hydroxysteroid dehydrogenase type 3 enzyme to reduce androgen synthesis, while enzalutamide (Enza) is a non-selective AR antagonist. Inhibitors of polycomb group protein 2 (EZH2) complex such as tazemetostat (Taz) are also potential antitumor agents in metastatic castration-resistant PCa (mCRPC) and AR translocation-positive prostate cancer (ATPC) models. Several studies have shown that EZH2 inhibition combined with abiraterone and enzalutamide improves clinical outcomes in mCRPC patients. In this study, we evaluated the potential of EZH2 inhibition in combination with androgen signaling inhibitors in preclinical models of prostate cancer.

**Methods and Materials**

- Gene expression profile of PCa cell lines representing PCa subtypes in two models
- In vitro Activity of Tazemetostat on proliferation
- Combination effects by in vitro assay
- Combination Effect Summary in Pretreatment Assay
- OS

**Results**

- Tazemetostat demonstrated modest antitumor activity as single agent in 22Rv1 (AR-V7+) mouse xenograft
- Tazemetostat enhances antitumor activity of ASIs in LNCaP mouse xenograft

**Conclusions**

- EZH2 inhibition resulted in time- and dose-dependent antiproliferative activity in PCa cells that are dependent on AR signaling or are of the neuroendocrine subtype
- Treatment of PCa cell lines with tazemetostat in combination with ASIs resulted in synergistic cell growth inhibition that was driven by apoptotic cell death and G0/G1 cell cycle arrest
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