# P73 Hydralazine, a new hope for CRPC patients?

Eur Urol Suppl 2019;18(8):e3141

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**Introduction & Objectives:** Prostate cancer (PCa) remains a worldwide concern due to its high incidence and associated mortality. Androgen Receptor (AR) is a central player in the pathogenesis of PCa and its expression promotes cell survival and growth, both in normal and cancer contexts. Nevertheless, as disease progresses, a portion of PCas will eventually lose AR protein expression and become dependent on alternative pathways. Previous work from our group showed that treatment of DU145 CRPC cell line (which does not express AR) with hydralazine (a DNMT inhibitor) restored AR protein expression while simultaneously decreased viability and promoted apoptosis. This implies that epigenetic mechanisms, such as methylation, are contributing factors to PCa disease progression and support the use of hydralazine as a therapeutic agent for AR-negative PCas in which AR protein loss is due to AR promoter methylation. Our aim was to assess the methylation status of regulatory regions of AR in hydralazine responsive (DU-145) and unresponsive (PC3) as well as normal (RWPE-1) cell lines in order to pinpoint the most relevant regions for this regulation. Furthermore, we combined hydralazine with antiandrogens, bicalutamide and enzalutamide, to establish the efficacy of a combined treatment approach.

**Materials & Methods:** The basal methylation profile of AR regulatory regions of CRPC (DU145 and PC3) and normal (RWPE-1) cell lines was assessed in WT cells. Three different regulatory regions were evaluated by bisulfite sequencing of at least 10 cloned alleles per region. Afterwards, the DU145 cell line was treated with different concentrations of hydralazine (1uM-50uM) and 5 clones were bisulfite sequenced. The same three cell lines were subjected to treatment with different concentrations of hydralazine (5uM, 15uM and 30uM) for 3 days followed by 3 days of treatment with antiandrogen enzalutamide or control vehicle. MTT assay was used to assess cell viability.

**Results:** Bisulfite sequencing of WT cell lines revealed that hydralazine responsive DU-145 cell line exhibits higher levels of AR methylation that the other two unresponsive (PC3 and RWPE) cell lines. Furthermore, hydralazine treatment of DU-145 promoted the demethylation of specific CpGs near the transcription start site that may be relevant for AR expression. Finally, we verified that 3 days of treatment with hydralazine followed by 3 days of enzalutamide was superior to hydralazine treatment alone in DU-145 cell lines.

**Conclusions:** Our results support the rationale of using hydralazine in combination with antiandrogens as a therapeutic tool for a subset of AR-negative PCa that harbour methylation of the AR promoter.