

## **Androgen Receptor DNA binding domain inhibitors to treat AR splice variant-driven prostate cancer**

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**Background:** Nearly all prostate cancer (PC) cells depend on androgen receptor (AR) signaling for growth. Resistance to novel PC drugs like abiraterone and enzalutamide appears to occur through ligand-independent AR signaling, often by expression of AR splice variants (AR-Vs) that lack the ligand binding domain (LBD). Thus, drugs that inhibit AR activity via domains other than the LBD may provide a way to inhibit the growth of drug-resistant PC. We discovered the first AR DNA-binding domain (DBD) inhibitor, pyrvinium pamoate, which functions in a non-competitive, ligand-independent manner by preventing the recruitment of RNA polymerase II to transcription start sites. Pyrvinium inhibits AR activity with low nanomolar potency and functions synergistically with competitive antagonists. Here, we further elucidate pyrvinium's mechanism of action and demonstrate its effectiveness in models of drug-resistant PC.

**Methods:** NMR was used to test pyrvinium binding to the AR DBD and mutational analysis of the DBD was used to define the region of binding. EMSA and ChIP were used to evaluate changes in DNA binding. AR-IP followed by mass spec sequencing was used to identify proteins that differentially bind to AR in the presence of pyrvinium. Additionally, RNA-seq was used to identify pathways affected by pyrvinium treatment. Functional studies of hits were conducted using over-expression and knock down in relevant PC cell lines. Efficacy was tested in enzalutamide resistant cells.

**Results:** NMR confirmed binding of pyrvinium to the AR DBD and mutational analysis indicated that residues K609 and P612 were important for activity. No significant changes in DNA affinity were identified. RNA-seq results suggest that AR and hormone pathways are the primary target of pyrvinium in PC cells but that the splicing machinery is also be affected. Mass spec results also demonstrate that pyrvinium affects the interaction between AR and splicing machinery, as well as many transcription and translation-related proteins. DDX17 was identified as an important mediator of pyrvinium activity. Comparisons to other DBD and N-terminal AR inhibitors demonstrate the superiority of pyrvinium. Finally, pyrvinium potently inhibited the growth of enzalutamide-resistant and AR-V driven PC cells.

**Conclusions:** Pyrvinium was the first identified AR DBD inhibitor and holds great promise for the treatment of AR-V driven PCs.

**Conflict of Interest:** None

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